

FLAVOUR FORMATION IN GHEE

A thesis submitted in partial fulfilment of the requirements for the
degree
of Doctor of Philosophy in Biochemistry
at the University of Canterbury

Angela E. Newton

2013

Abstract

Consumers place a high level of importance on flavour when assessing the acceptability of food. The flavour of dairy products can be affected by heating both during processing and by consumers during food preparation. Of particular importance to the flavour of heated dairy products is the highly complex network of Maillard reactions. Previously, emphasis has been placed on undesirable flavours generated through the Maillard reaction in dairy products and efforts have been made to minimise the formation of these flavours. However, beneficial flavours are also formed by the Maillard reaction. Dairy products such as ghee are formed by heating and are characterised by their unique flavours; these flavours are generated by the Maillard reaction.

The objective of this thesis was to unravel the factors that influence the Maillard and caramelisation reactions that occur during the cooking of ghee. Particular focus was placed on the impact that the structure of the cream had on the reaction, and how manipulation of these parameters could provide an avenue for the control of the Maillard reaction.

The development and validation of model reaction systems for the Maillard and caramelisation reactions involved the variation of parameters including cooking time, temperature, pH, phosphate buffers and salt. A group of eleven compounds including acetic acid, furfural, 2-acetylfuran, butyrolactone, 2(5H)-furanone, furfuryl alcohol, maltol, 2-acetylpyrrole, hydroxymaltol, hydroxymethylfurfural (HMF) and dihydro-4-hydroxy-2(3H)-furanone (DHMF) were monitored to determine the impact of these parameter changes. These results provided a starting point to assess the impact of food structure on these reactions. To assess the impact of food structure a series of matrix structures were designed starting from an aqueous matrix. The first component that was added to the aqueous matrix was fat to generate a two phase structure. Emulsion structures were then formed from the two phase structure using emulsifiers and high pressure homogenisation.

Analysis of the volatile compounds formed as the matrix structures were altered was carried out using headspace solid phase microextraction/ gas chromatography mass spectrometry (SPME/GCMS). Results indicated that fat is a key structural component in flavour generation via the Maillard reaction. This could have implications for low fat foods where the flavour

developed during cooking is important. The addition of fat indicated a significant impact on the Maillard reaction with a less significant impact seen on the caramelisation reaction.

The formation of two emulsions with inverted structures provided a means to alter the ratio of volatile compounds in the cooked samples. The oil in water emulsion generated a volatile compound profile similar to that of the fat containing matrix, whereas the water in oil emulsion produced a different ratio of these same compounds.

The results reported in this thesis shed some light on the relationship between food structure and flavour formation during the cooking of milk fat emulsions. These structures will create future opportunities to manipulate the structure of food to control flavour formation.

Acknowledgments

I would like to thank my supervisors for their constant help, encouragement and wonderful supervision throughout my PhD. Thank you to Dr Juliet Gerrard for her prompt email responses, awesome suggestions, superb proof reading and positive reassurances when I was having moments of doubt and reminding me that it was ok to go on holiday without my thesis. A huge thank you to Dr Paul Andrewes for putting up with me on a daily basis, answering my endless questions, helping to fix/troubleshoot the GCMS when things didn't go to plan, and for reminding me of the big picture when I was heading off on a tangent. Thank you also to Dr Antony Fairbanks for your mechanistic brilliance and patience answering my questions and to Dr Matt Golding for your expert emulsions knowledge and valuable answers to my many questions.

To everyone who was part of the former biotransformations and flavour team, and the current food chemistry team, thank you for your support. Special thanks to Andrew Broome, Michael Loh, Ivan Simpson, and Skelte Anema for your assistance with lab equipment and ensuring experiments ran smoothly. Thank you to Tim Coolbear for his excellent proof reading skills and to Lucy Justice for your statistics assistance.

Thank you to the Gerrard research group at the University of Canterbury for accepting me as one of the group even though you probably all wondered at one time or another if I actually existed. Thank you to Dr Justine Cottam who has become a great friend over the course of my PhD: thank you for all of your advice, encouragement and time passing conversations when we were sharing an office space (corner of the lab). I am grateful for the assistance from Dr Susie Meade with travel arrangements, accommodation bookings and paperwork wrangling to ensure that working across two locations ran smoothly.

Of course this PhD would not have been possible without the support and understanding of my friends and family. Thank you to my Fightshop family particularly Stew, Tash, Paula, Caitlin and Sue, for accommodating my ever changing schedule, missed training sessions, my often distracted state of mind and for listening to me ramble about things you didn't understand!!! I could never have won a New Zealand title while doing my PhD without you!! To all my other friends, thank you for always being there when I needed a laugh, a wine and a mental break from my PhD.

I am extremely grateful for the constant support of my parents, Vicki and Colin. Thank you for always encouraging me to set high goals and chase my dreams, the newspaper clippings, cups of tea, for being interested in things you don't understand and finding mistakes others had missed. Everything

you have done for me was greatly appreciated. Thank you to my in-laws, Ian and Jan for understanding when I stayed at home to work or I brought my PhD to stay.

Finally, to my darling Mike who always understood when I was tired, busy, cranky and horrible to live with, and thank you for telling me that I was none of those things! Thank you for always listening and putting things in perspective when I thought the world was ending, for our wonderful weekends away without my thesis and for agreeing to honeymoon in Europe so I could present at a conference, it was the most amazing trip!! I could not have survived my PhD without your love and support. 'Love you always'

This work was co-funded by Fonterra Co-operative Group Ltd and the New Zealand Ministry for Science and Innovation (Project DRIX0801, Dairy Ingredient Solutions for Nutritional Vitality).

Table of Contents

Abstract	i
Acknowledgements	iv
List of abbreviations	x
Chapter 1 Flavour formation in ghee – background information	1
1.1 Introduction	1
1.2 Maillard reaction	2
1.3 Factors influencing the Maillard reaction	5
1.4 Studying the Maillard reaction with a view to flavour control	13
1.5 Flavours in dairy products	17
1.6 Mechanistic pathways	23
1.7 Conclusion	25
1.8 Thesis aims/chapter outline	26
1.9 References	28
Chapter 2 Volatile compound analysis	37
2.1 Introduction	37
2.2 Analysis of volatile compounds	37
2.3 Standard samples	40
2.4 Gas chromatography optimisation	42
2.5 Solid phase micro extraction (SPME) optimisation	48
2.6 Mass spectrometry	51
2.7 Data analysis	52
2.8 Compound identification	55
2.9 Reproducibility	56
2.10 Error calculations	57
2.11 Matrix interferences	58
2.12 Conclusion	61
2.13 References	62

Chapter 3	Ghee	65
------------------	-------------	-----------

3.1	Introduction	65
3.2	Traditional ghee cooking methods	65
3.3	Modelling the cooking of ghee	68
3.4	Influence of time and temperature	69
3.5	Comparing high throughput cooking to wok ghee	73
3.6	Structure of starting materials	75
3.7	Conclusion	77
3.8	References	78

Chapter 4	An experimental model for the Maillard and caramelisation reactions	79
------------------	--	-----------

4.1	Introduction	79
4.2	Modelling the Maillard and caramelisation reaction in cream	79
4.3	The differences between the Maillard and caramelisation reactions	81
4.4	Influence of time and temperature	85
4.5	Influence of pH on the Maillard and caramelisation reactions	89
4.6	Other components of cream that influence the Maillard reaction	95
4.7	Conclusion	99
4.8	References	100

Chapter 5	The role of fat in the Maillard and caramelisation reactions	103
5.1	Introduction	103
5.2	The influence of fat on the Maillard reaction	104
5.3	The influence of fat on the caramelisation reaction	107
5.4	Aqueous concentration effect	111
5.5	Reaction temperature and evaporation	112
5.6	Participation of fat in the Maillard reaction	114
5.7	The partitioning effect of the two phase reaction matrix	115
5.8	Conclusion	116
5.9	References	118
Chapter 6	The role of emulsion structure in the caramelisation and Maillard reactions	120
6.1	Introduction	120
6.2	Formation of emulsions	122
6.3	Impact of emulsifiers	123
6.4	Influence of emulsion structure	126
6.5	Conclusion	139
6.6	References	141
Chapter 7	Conclusion	143
7.1	Future work	147
7.2	References	148

Chapter 8	Materials and methods	152
8.1	Materials	152
8.2	Gas chromatography mass spectrometry	152
8.3	General methods	158
8.4	Ghee	158
8.5	Aqueous experimental model system	160
8.6	The addition of fat to the aqueous model system	163
8.7	The influence of emulsion structure	164
8.8	References	168
Appendix A	Proposed mechanisms	171
Appendix B	Fonterra product bulletins	180
Appendix C	Statistical analysis and tables	186

List of Abbreviations

°C	degrees Celsius
°Cmin ⁻¹	degrees Celsius per minute
µg/kg	micrograms per kilogram
µm	micrometre
AMF	anhydrous milkfat
ANOVA	analysis of variance
Aq	aqueous
AS	autosampler
a_w	water activity
B/B	butter/buttermilk
C18	eighteen carbon length chain
C4	four carbon length chain
CB	cream butter
CE	capillary electrophoresis
d(0.1)	the diameter under which 10 % of particles exist in the sample
d(0.5)	median particle diameter
d(0.9)	the diameter under which 90 % of the particles exist in the sample
D[3,2]	surface area moment mean
D[4,3]	volume moment mean
DC	direct cream
DF300	dairy flavour 300 (standard reference compound)
DHHF	dihydro-4-hydroxy-2(3H)furanone
EI	electron ionisation
eV	electron Volts
FAME	fatty acid methyl ester
gal	galactose
GC	gas chromatography
GCMS	gas chromatography mass spectrometry
HLB	hydrophilic lipophilic balance
HMF	hydroxymethylfurfural
Hz	hertz
LCMS	liquid chromatography mass spectrometry

logP	partitioning coefficient
M	molar
MB	milk butter
mg	milligrams
Min	minutes
ml	millilitre
mM	millimolar
mm	millimetre
mML ⁻¹	millimoles per litre
MS	mass spectrometry
N	refractive index
n/a	not applicable
NMR	nuclear magnetic resonance
o/w	oil in water emulsion
P	partial pressure of water in the system
PCA	principle components analysis
PDMS/DVB	polydimethylsiloxane/divinylbenzene
PGPR	polyglycerol polyrinocleate
pK _a	acid dissociation constant
P _o	partial pressure of pure water
PS	prestratification
Psi	pounds per square inch
rpm	revolutions per minute
SDS-PAGE	sodium dodecyl sulfate – polyacrylamide gel electrophoresis
SIM	single ion monitoring
SPME	solid phase microextraction
TP	two phase
UT13500	ultraturrax at 13,500 rpm
UT20000	ultraturrax at 20,000 rpm
UT8000	ultraturrax at 8,000 rpm
UV	ultraviolet
w/o	water in oil emulsion
w/v	weight for volume
w/w	weight for weight

1. Flavour formation in Ghee

This thesis investigates the role of emulsion structure on the Maillard and caramelisation reactions that occur during the cooking of ghee, with a view to controlling these reactions in order to manipulate flavour formation.

The introduction provides an overview of the Maillard reaction occurring between a reducing sugar and an amino group of a protein or amino acid and the caramelisation reaction of the reducing sugar alone in dairy products and model systems, along with various factors that can influence these two reactions.

1.1 Introduction

The flavour of dairy products can be particularly important to consumers (1). Milk that is fresh and of high quality typically has a characteristically mild flavour, and consequently any deviation in flavour will be obvious. Whilst a major source of flavour components is the milk itself, the degradation of milk constituents during processing can also have a significant impact on the final flavour profile of dairy products.

The quality of many foods, including dairy products, is affected by the Maillard reaction (2), which becomes more significant when those foods are heated (3). This highly complex network of reactions can produce an attractive taste, appearance and aroma for the consumer (2, 3) or it can develop undesirable colours, off-flavours and potentially harmful reaction products (2, 4-6). Changes to the functional properties of milk proteins can also occur (7). In the context of dairy foods, these undesirable flavours are of concern in both the production and storage of UHT milks and dried milk powders (6): for this reason minimisation of Maillard chemistry in dairy products has been a priority in the field. However, these same flavours are desirable in ghee (8, 9), brown butter, Ryazhenka Kefir (10), grilled cheese, condensed milk, toffees, fudges, dulce de leche (11) and milk chocolate crumb (12, 13). Thus, depending on the desired product quality, both minimising and enhancing the progress of the Maillard reaction is of interest to dairy food processors.

Model systems have been employed to understand the chemistry of the Maillard and caramelisation reactions and how the environment in which the reaction occurs can influence the reaction outcomes. There has been a focus has been on microemulsions that could provide avenues for the control of volatile flavours (14-19).

1.2 Maillard reaction

The Maillard reaction is named after Louis Camille Maillard, who first described the reaction that occurs between an amino group and a carbonyl moiety (20, 21) in 1912. The amino group can be part of an amino acid, peptide or protein, any of which can react with a variety of carbonyl-containing compounds, typically sugars (such as glucose, fructose or lactose) or their derivatives, or fat breakdown products (22, 23). The Maillard reaction is a thermally-induced reaction occurring most often at high temperatures. In most dairy products the most abundant carbonyl compound will initially be the reducing sugar lactose (or its hydrolysis products, glucose and galactose) and the amino groups could be derived from either casein or whey proteins, which contain amino acid residues that have been shown to be reactive to Maillard chemistry (22), e.g., lysine. Other sources of amine groups could include amino phospholipids (24) or free amino acids (25), whilst milk fat provides another source of carbonyl compounds (23).

There are multiple stages to the Maillard reaction (figure 1.1). The initial stage (figure 1.2) is typically a condensation reaction between the sugar and an amine to form an unstable glycosylamine (imine). This glycosylamine can then undergo rearrangement to form the Amadori compound, an aminodeoxyketose. This Amadori compound can react along multiple parallel pathways to form advanced Maillard reaction products, a selection of which are illustrated in figure 1.3 (21, 26). Compounds formed by the Maillard reaction in dairy products commonly include furfural, 2-acetylfuran, furfuryl alcohol, hydroxymethylfurfural (HMF) and maltol (figure 1.4). For a detailed treatment of the many and varied reaction pathways of Maillard chemistry see Ledl and Schleicher (20), Nursten (21), and Gerrard (27).

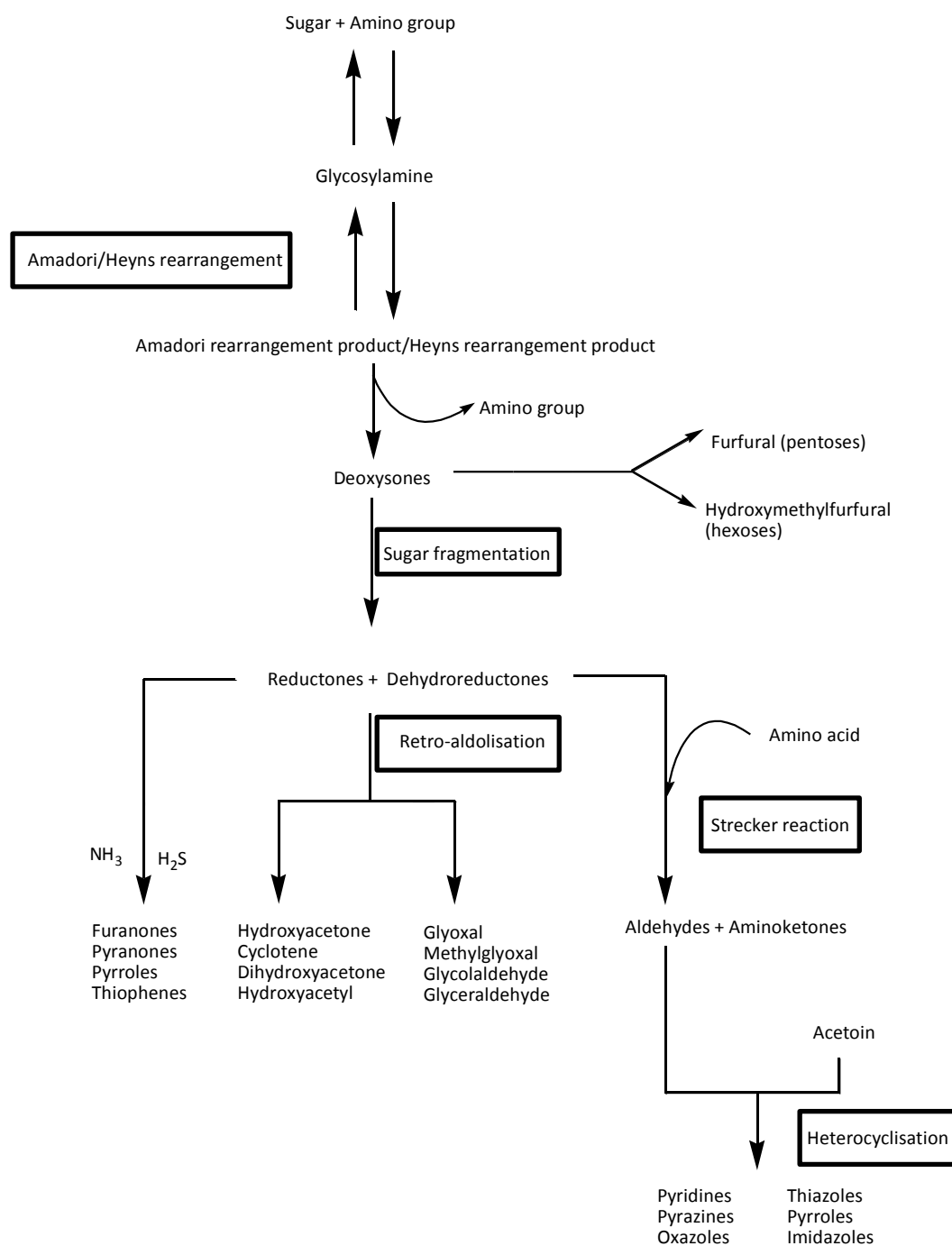


Figure 1.1 Overall Maillard reaction schematic. Adapted from van Boekel (2006) (3).

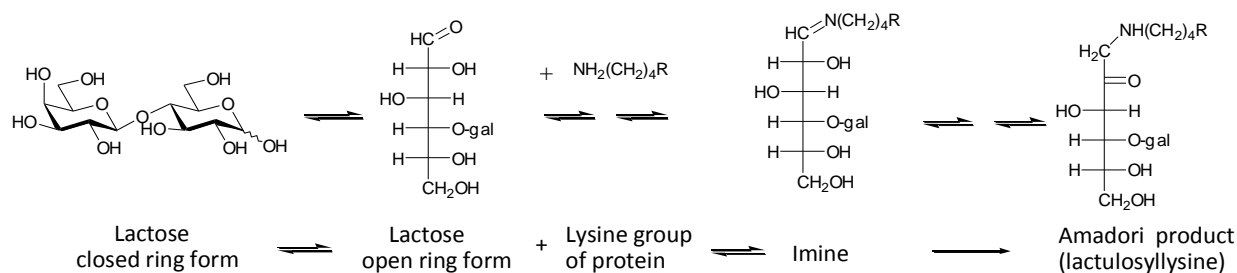


Figure 1.2 Early Maillard reaction products of lactose (6) (gal, galactose; R, protein chain).

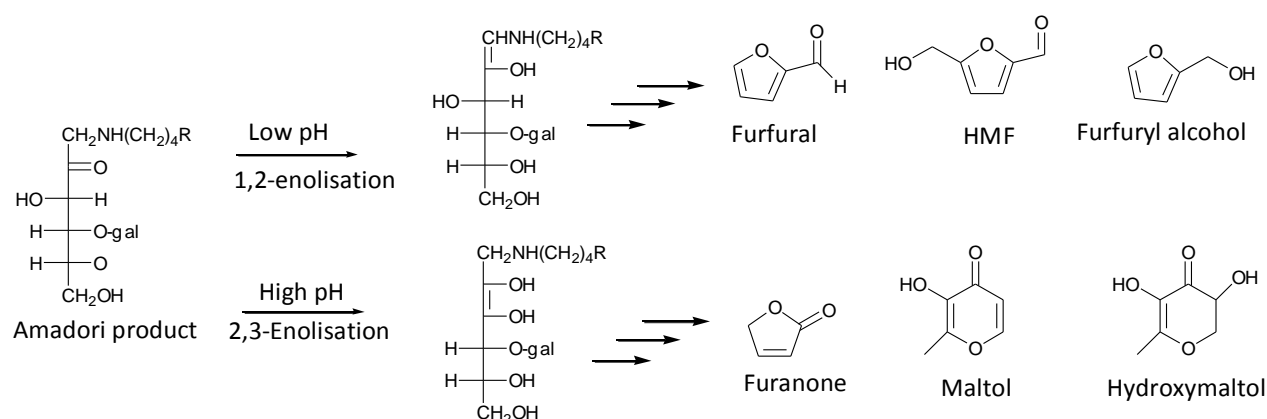


Figure 1.3 Influence of pH on the enolisation pathways (6) (gal, galactose; R, protein chain).

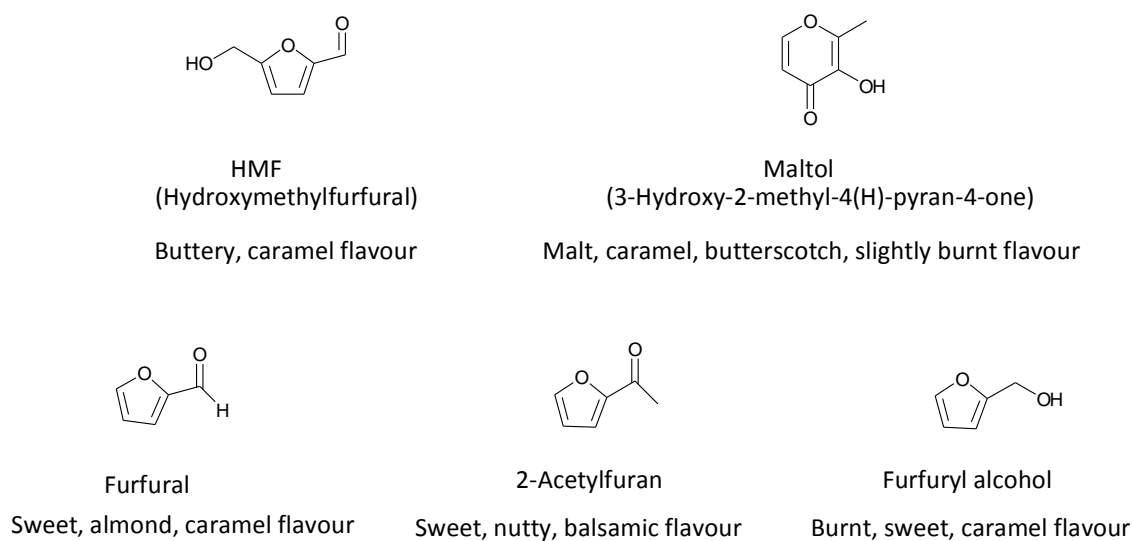


Figure 1.4 Common Maillard reaction compounds responsible for flavour in dairy products (1, 3, 6, 10, 28-31).

1.3 Factors influencing the Maillard reaction

The Maillard reaction system is comprised of multiple reaction pathways, each of which can be favoured relative to alternate pathways under different conditions, making optimisation of the reaction under a given set of conditions very challenging (32). In a food system, the Maillard reaction is dependent on several factors: the type of sugar, the type of amino acid residue, the temperature, the pH, the water activity, the presence of any buffers, the oxygen availability and the nature of the food matrix (2, 3, 27, 32). These factors need to be considered during both food processing and food storage (3).

The following sections outline the impact of these different factors with reference to dairy systems.

1.3.1 Type and identity of sugar

One of the reactant factors affecting the Maillard reaction is the type of sugar and the nature of its breakdown products. Aldose sugars will react differently from ketose sugars (33). Aldoses such as glucose, mannose, galactose and ribose give rise to the Amadori intermediate, whilst the ketoses such as fructose, tagatose, ribulose and psicose give rise to the corresponding Heyns compound (33). Each individual sugar will exhibit a different rate of reaction (34) with monosaccharides reacting faster than disaccharides. This is in part due to the kinetics of the ring opening equilibrium. From each of these intermediate products (Heyns and Amadori) the mechanism moves towards different carbonyl compounds (35) and advanced reaction products, the formation of which can significantly alter the flavour and aroma profile. The yields, product compositions and rates of these steps are largely dependent on the pH (35): slightly acidic, neutral and/or slightly basic conditions will all yield different products. Most dairy products will contain significant quantities of lactose, a disaccharide of glucose and galactose (6), but ascorbic acid is also present that can itself react with amines to form Maillard compounds (36, 37). The addition of other sugars to a dairy food product might significantly alter the flavour profile.

Lactose participates not only in the Maillard reaction as a reducing sugar but also undergoes degradation by a second pathway. At high temperatures (usually >100 °C) lactose is isomerised to lactulose (figure 1.5) that subsequently degrades to galactose, formic acid and a range of C5/C6 compounds (38, 39). These transformations, in which amino compounds are not involved, form a subset of chemical browning reactions referred to as caramelisation reactions (figure 1.6). The rate of transformation and the rate of the caramelisation reaction are dependent on the type of sugar in a similar manner to the Maillard reaction (39).

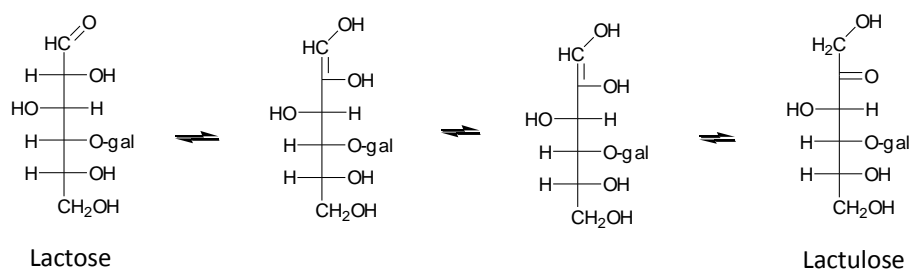


Figure 1.5 Isomerisation of lactose to lactulose *via* the Lobry-de Bruyn van Ekenstein transformation (38, 40, 41).

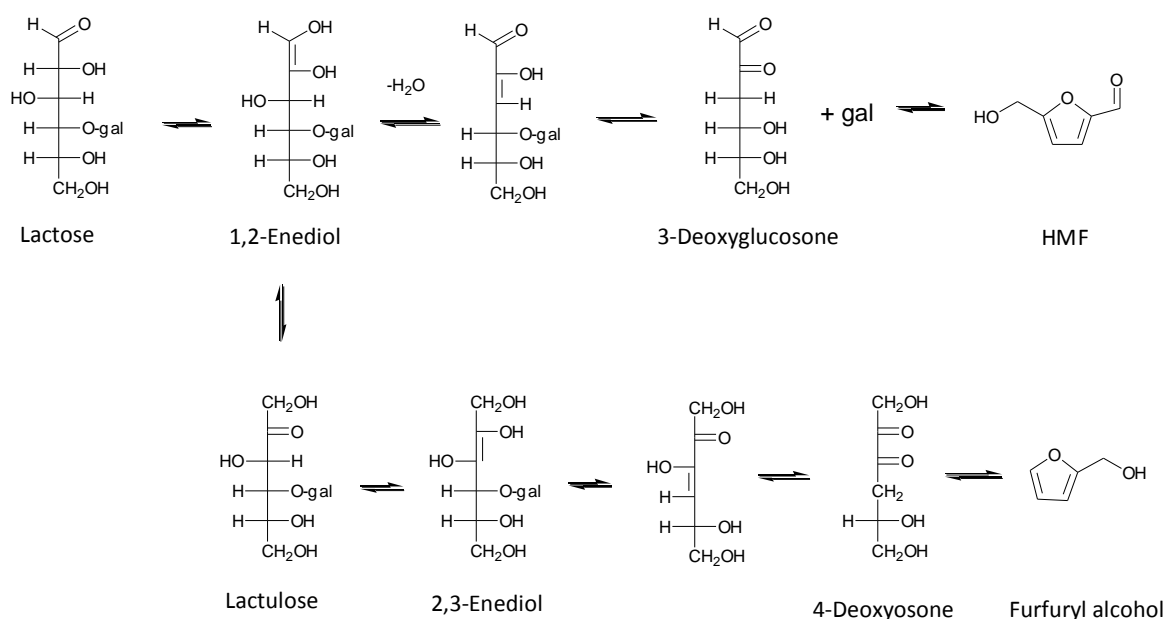


Figure 1.6 Initial stages of the caramelisation reaction leading to the formation of 4-deoxysone and 3-deoxyglucosone. The caramelisation reaction of lactose can form a range of compounds including HMF and furfuryl alcohol (38) (gal = galactose).

1.3.2 Type and identity of amine

The involvement of different amino groups in the Maillard reaction will lead to a range of alternate flavour profiles as a result of the different compounds that can be formed. To illustrate this table 1.1 (42) shows the range of flavours possible by changing the amino acid heated with glucose, and a selection of compounds that are responsible for these flavour profiles is shown in figure 1.4. Dairy proteins contain a range of amino acids, some of which have residues able to participate in the

Maillard reaction without breakdown of the protein. Hydrolysis of the protein also results in increased amino group availability for the Maillard reaction.

Table 1.1: Possible flavours arising from heating different amino acids with glucose under various conditions (42).

Amino acid	Flavour of product formed on heating with glucose
Alanine	Fruity, flowery, sweet
Arginine	Bitter, sour, fruity
Aspartic acid	Fruity, sweet
Cysteine	Sulfur, meaty
Glutamic acid	Sour
Glycine	Caramel, sweet, flowery
Histidine	Sour
Isoleucine	Burnt, caramel
Leucine	Burnt, caramel
Lysine	Pleasant/sweet, caramel, cardboard, herbal tea
Methionine	Potatoes, prawn crackers
Threonine	Sweet, fruity, astringent
Serine	Fruity, sweet
Proline	Fruity, bitter
Phenylalanine	Flowery, almond, bitter
Tyrosine	Fruity, flowery, tea-like
Valine	Caramel, biscuit, malty, chocolate, bitter

1.3.3 pH and buffers

The yields, product compositions and rates of many of the Maillard and caramelisation steps are influenced by the pH of the reaction system (35). Reaction of the same components under slightly acidic, or neutral, or slightly basic conditions will all yield different products. The ring opening of reducing sugars is one variable that explains this dependence. The concentration of the open chain form of the sugar can play a role in the overall reaction rate as it affects the reactivity of the sugar (32). In cases where the equilibration to the open chain form of the sugar is the rate limiting step (32), the rate of the initial stages of the Maillard reaction will correlate with higher equilibrium concentrations of the open chain form of the sugar (figure 1.7). The sugar can also isomerise (41, 43-

45) and degrade to Maillard-reactive dicarbonyl compounds, such as methylglyoxal (46), carboxylic and organic acids (38).

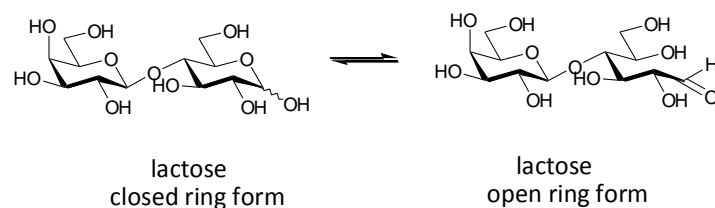


Figure 1.7 Ring opening equilibrium of lactose.

The pH of the solution also determines the protonation state of the amine group (figure 1.8), which in turn will alter the rate of the initial reaction, that yields the Amadori product (figure 1.2). The protonated and non-protonated forms of the amine compound are in a pH dependent equilibrium, with a higher portion being protonated at low pH. The pK_a of the protonated form of the reacting amino compounds will therefore also influence the optimal pH for the early Maillard reaction, since only the non-protonated form is reactive. At pH 7, only approximately 1% of amine groups are non-protonated, which explains one facet of the dependence of reaction rate on the pH of the system (32).

The pH is also important after the formation of the Amadori compound, as it can determine which pathway the reaction then predominantly follows: e.g., 1,2-enolisation has been found to predominate at low pH, whereas a 2,3-enolisation pathway is preferred at a high pH (figure 1.3) (6). These different pathways then give rise to different products, e.g., furanone *via* 2,3-enolisation and furfural and HMF *via* 1,2-enolisation (6). Various mechanisms have been proposed that lead to a range of Maillard reaction compounds from the Amadori compound, as summarised in figure 1.3.

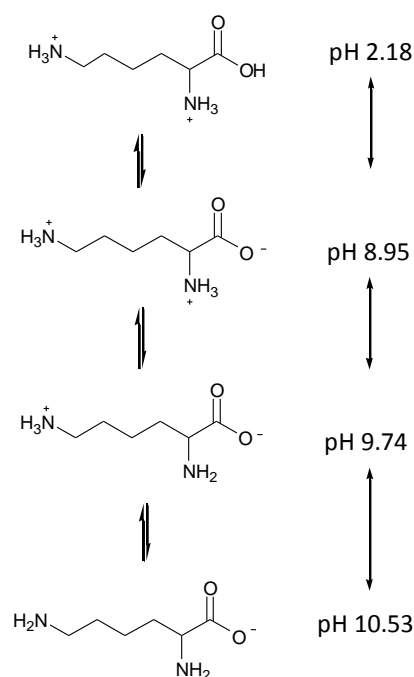


Figure 1.8 Changes in protonation of lysine with changes in pH (47).

The presence of buffering agents in the reaction system will alter the rate of the Maillard reaction (48). Phosphate buffers have been seen to have a catalytic effect, leading to an increase in reaction rate. The interaction of the phosphate buffer increases the rate of reaction through the initial stages of the Maillard reaction as shown in figure 1.9. Other buffers, including citrate buffers, do not have this same effect (48). The concentration of the buffer is also important (49).

The influence of phosphates on the Maillard reaction has been studied using phosphorylated glucose (50). Phosphorylation of the glucose leads to an increased rate of reaction due to the phosphate providing a good leaving group. The presence of the phosphate group also allowed for some degree of control over the Maillard reaction as the phosphate group provided additional stabilisation for some reaction intermediates and prevented some nucleophilic pathways by blocking and sterically hindering the required reaction sites (50).

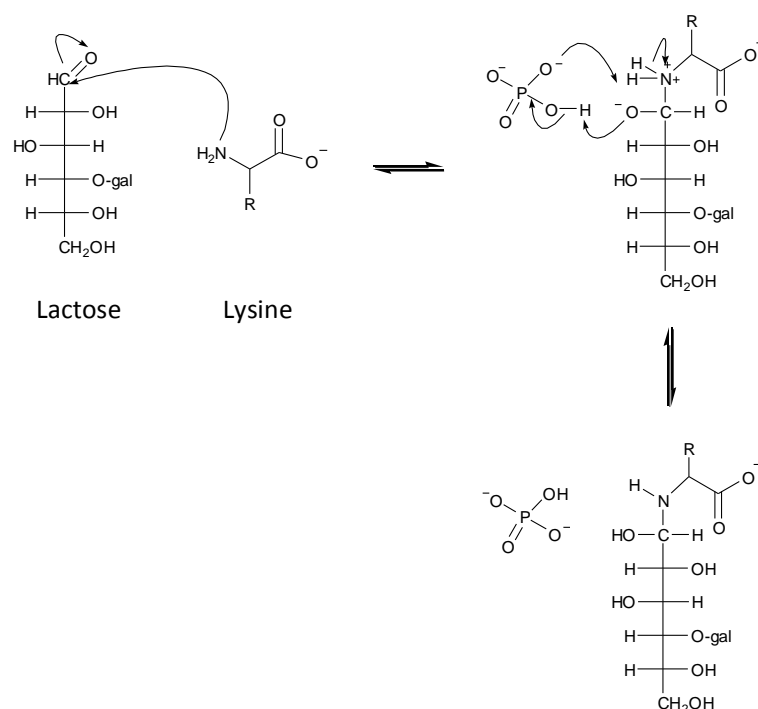


Figure 1.9: Proposed catalytic effect of the phosphate anion on the Maillard reaction of lactose and lysine (50).

1.3.4 Temperature

The reaction temperature is an important parameter, particularly above 100 °C. It is the driver behind the initiation of the Maillard reaction that is most often seen in food systems cooked at elevated temperatures (6). Not only the overall reaction rate, but the relative rates of different reaction pathways are also dependent on temperature (32). Generally, the amount of volatile compounds found in a sample increases with an increase in temperature (51). Pasteurisation and UHT processes are conducted at elevated temperatures, as is the manufacture of products such as ghee and dulce de leche. Other cooking requirements such as for pizza cheese and grilled products, also results in exposure to elevated temperatures (9, 11, 52-54).

Consideration should also be given to the difference between the cooking temperature and the actual food temperature (55). Due to water evaporation, temperature gradients, and thermal conductivity, the temperature of the food can be significantly less than the cooking temperature during the early heating stages. For example, this difference between the set temperature and the actual temperature means that while an oven may be set to cook at 130 °C for 30 minutes, the actual temperature of the food may only be 90-100 °C until the water has evaporated, at which point the temperature may rise to the set temperature. This also implies that the temperature

throughout a food may not be uniform (55). This means that the extent of the Maillard reactions in a cooking process may be less than expected from simple considerations of the cooking temperature.

When food is cooked in an open system, flavour loss, with vapours coming off the food, is apparent from the strong aromas of cooking foods. As the temperature is increased more vapour is likely to be driven from the food (especially steam). The net effect on the loss of individual flavour compounds is complex. However, it is the volatile compounds remaining in the food that are contributing to the flavour of food. In general, the compounds remaining in the food, after cooking, increase with higher cooking temperatures regardless of an open or closed system is used.

1.3.5 Lipid degradation compounds

All dairy products contain lipid components as either part of the matrix (cream, milk, cheese) or as a major component (butter, ghee), placing potentially different levels of importance on the flavour imparted by the lipid. Carbonyl compounds are formed during the oxidation of lipids and are also intermediates of Maillard reaction products (56). The degradation and oxidation of lipids are important to the flavour of food (23, 57), as these breakdown products can react with other Maillard reactants, leading either to deterioration or to improvement of food quality. Compounds such as methyl ketones, aldehydes and free fatty acids (23) are formed when fats are heated. These volatile compounds can then go on to react with amino acids and/or Maillard reaction products. A greater degree of browning and volatile compound formation was seen in model systems that contained lipid oxidation products when compared with a model system in which there were no lipid oxidation products (58), demonstrating that the lipid oxidation products took part in the Maillard reaction. Lipid oxidation reactions can also occur prior to the heat treatment and are usually favoured under acidic conditions in the presence of oxygen (59).

Lipid degradation can lead to the production of rancid flavours that can be of particular concern in dairy products, especially those with limited shelf life or a high fat content. However, the Maillard reaction may reduce this phenomenon by removing rancid compounds *via* both the reaction itself and the ability of some Maillard reaction products to exert an antioxidant effect, which could prevent rancid flavour compounds from being produced (23, 60, 61).

1.3.6 Water Activity

The water activity (a_w) of a reaction system is an index that describes the availability of water to participate in reactions. It is defined by the equation $a_w = P/P_o$, where P is the partial pressure of the water in the system and P_o is the partial pressure of pure water (62). The Maillard reaction occurs optimally when a_w values are between 0.5 and 0.8 (63, 64).

Water activity varies widely between dairy products, ranging from very high values in products such as milk ($a_w > 0.95$) (65), to very low values in products with low water content such as milk powder ($a_w < 0.1-0.4$) (66) and ghee. There are multiple possible dehydration processes along with many of the Maillard reaction pathways that will each generate additional water molecules. In a system with excess water (dilute solution) a high a_w will reduce the rate of the biomolecular steps of the Maillard reaction due to reactant dilution. If the water activity (a_w) is lowered, reactants may begin to lose mobility that is essential for the reaction to occur, thus resulting in a lower rate of reaction (64). The low water activity of milk powder slows the Maillard reaction to minimise the formation of detrimental flavour compounds during storage (67).

1.3.7 Influence of metals

The Maillard reaction can also be influenced by the formation of metal complexes between amino acids and available metal ions. The reaction can be catalysed by copper (Cu^{2+}) and iron ($\text{Fe}^{2+/3+}$) but inhibited by manganese (Mn^{2+}) and zinc (Zn^{2+}). Calcium (Ca^{2+}) has also been shown to delay the Maillard reaction by forming complexes with certain sugars (68). Dairy products contain calcium and traces of other metals that can complex with the amino acids and so influence the reaction rate. Lipid oxidation can also be catalysed by copper and iron (69) to generate reactive dicarbonyl compounds.

1.3.8 The impact of emulsion structure

Cream and butter have emulsion structures consisting of either water or oil droplets dispersed in a continuous phase. The emulsion structures are described as water in oil (w/o) or oil in water (o/w) emulsions and are characterised by a large particle size, lack of transparency and are kinetically stable (70). In contrast, microemulsions have a uniform structure, a low viscosity and are thermodynamically stable. Typically, they have a particle size in the order of nanometres (5-100 nm) (70).

In an aqueous reaction system the reactants are free to interact with each other; whereas in a structured fluid they are confined to droplets (emulsions/microemulsions) or channels (a bicontinuous phase) (19). When the concentration of reactants was kept constant across aqueous, emulsion and cubic (bicontinuous) systems, the rates of product formation were found to be different (14-18). It was proposed that the effective partitioning of the reactants within the emulsion structure created a localised concentration gradient that increased the proximity of reactants in certain system locations, thereby increasing the frequency of collisions between reactants and so the overall reaction rate (19). The localised concentration gradient arose because although the overall amount of each reactant had been kept the same, the phase that it occupied had a smaller volume and therefore the reactant concentration was increased within that phase.

1.4 Studying the Maillard reaction with a view to flavour control

1.4.1 Model studies

A considerable amount of information about the Maillard reaction has been collected using model studies, rather than within the complexity of the reaction systems in food (3, 20). The food matrix itself can have a large effect due to pH, water content/activity and other influencing factors, as discussed above. Models attempt to predict the rates of formation of Maillard reaction products as a function of these influencing factors and can be empirical (mathematical) or mechanistic (based on the knowledge of the chemical reaction and system) (71). The use of a model can allow testing of mechanistic predictions and provide insights by simplifying a problem to its basic components (71). However, the translation to real food systems can be problematic. When the experiments are carried out on real food the results can be conflicting as there are confounding factors influencing the results (71). Typical model systems for the Maillard reaction contain an individual amino acid and sugar – the simplest starting materials. Such studies/models can be extended to proteins and sugars (26) and further layers of complexity such as emulsion structure can be added to the model system in a controlled manner as the complexity begins approaching that present in the actual food system.

There are various parameters to consider when setting up a model system: the sugar, the amino compound, the reaction matrix (aqueous, lipids, emulsion) and the reaction conditions (pH, water activity, time, and temperature). Various experimental temperatures and times have been used in model studies: low (37-80 °C) (14, 51, 64) moderate (100-150 °C) (34, 37, 72-74) and high (>200 °C) (73), with times for experiments ranging from minutes to hours (17, 72, 75, 76).

1.4.2 Examples of models and their uses

There are a wide range of model systems that could be applied to dairy products, depending on the nature of the study being carried out (33, 61, 77-80). These studies typically focus on how the reaction compounds resulting from the Maillard reaction change when parameters associated with the system are changed rather than addressing the impact on flavour of the products (81-84).

A simple model that has been used to study the Maillard reaction in dairy systems was comprised of a monosaccharide (e.g. glucose, galactose, fructose, tagatose) with casein (33) in an aqueous phosphate buffer. This system contained the sugar (150 mM) with sodium caseinate (3% w/w) in aqueous phosphate buffer (0.1 M, pH 6.8), giving a molar ratio of sugar to lysine residues of 10:1. The model reaction system was heated to 120 °C. The purpose of this model was to demonstrate the differences in reaction mechanisms between an aldose sugar (glucose, galactose) and a ketose sugar (fructose, tagatose) in the same system, under the same conditions (33). The sugars were also heated in the system in the absence of sodium caseinate to allow the separation of caramelisation products and pathways from those that were Maillard products and pathways. The study demonstrated that there was a difference in the results for aldose sugars and ketose sugars. The researchers observed that the ketoses had a faster rate of reaction than the aldoses (33) and that these sugars also followed different Maillard reaction pathways that led to different colours, flavours and nutritive damage in the final products. This model system and the reaction conditions are similar to those involved in heating milk (pH 6.7), with caseins, sugars, and high temperature leading to coloured and flavoured products. Thus there are similarities between the products formed in this system and those reportedly formed in heated milk (33, 38). These product similarities include furfuryl alcohol, acetic acid, formic acid and various Maillard intermediates (33) identified in the model system that were identified in milk alongside HMF and furfural (38). The addition of lactose to the list of sugars studied would provide a better model of the reaction occurring in dairy products at high temperatures. The degradation of lactose in milk was studied using a model system consisting of lactose and sodium caseinate dissolved in a milk salt solution to establish the degradation pathways (38).

Model systems have been developed to allow the identification of compounds and development of methods for tracking heat treatment markers in milk. A homogenised lactose and sodium caseinate solution has been used to model milk in an effort to develop an assay to detect HMF formed during heat treatment (85). This study was also used to look at the impact of fat content on formation of HMF as a heat marker in milk in the same study.

A simple model system of lactose and lysine has also been used to develop a method to identify heat treatment markers in milk products (35, 85). The yield, composition and rates of the initial Amadori compound formation depended mainly on the system pH. To allow control over the pH, buffers such as sodium bicarbonate (NaHCO_3) (76) are often used. The desired pH influences which buffer is used; care must be taken to avoid using buffers that contain reactive amine moieties (such as Tris), but sodium acetate and sodium phosphate can successfully be used in conjunction with HCl or NaOH to adjust the final pH (86). The lactose-lysine model system (35) utilised aqueous solutions of lactose and lysine without pH control. The change in pH was used to monitor the progress of the reaction, together with relative antioxidative efficiency and optical density as the brown colour developed (35). Relative antioxidative efficiency is a parameter that has been measured in numerous studies involving the Maillard reaction (75, 76, 87, 88) and has potential relevance in dairy systems, such as chocolate, grilled cheese and ghee, where advanced Maillard chemistry is likely to generate a large number of anti-oxidant compounds. These studies were focused on finding the flavour compounds that form and discovering markers for the Maillard reaction, rather than the impact of these compounds on the flavour of the product.

Models have also been used to study specific compounds generated by the Maillard reaction and the mechanistic pathways by which they are formed, using isotope-labelled starting materials. In a dairy system this method could be used to investigate formation pathways of flavour compounds found in heated dairy products such as furfural, and flavours associated with off-flavours. Figure 1.10 illustrates the formation of furan and the different positions of the label in the final product. Isotope positions in the final product are dependent on the mechanism of formation (72). The formation of furan and methylfuran in model systems and food systems has been studied using isotopically labelled ascorbic acid (37) and ^{13}C labelled sugars (72). These models were used both under dry roasting conditions and aqueous conditions, and food systems were monitored by spiking pumpkin puree with the labelled compounds and then heating under the same conditions as were used in the model system (72). This allowed the influence of a food matrix on the formation mechanisms to be assessed and clearly demonstrated the relative importance of the pathways highlighted below and that the furan formation from sugars and amino acids represented only a minor route. Other routes involve recombination of fragments originating from sugar and protein fragments (72).

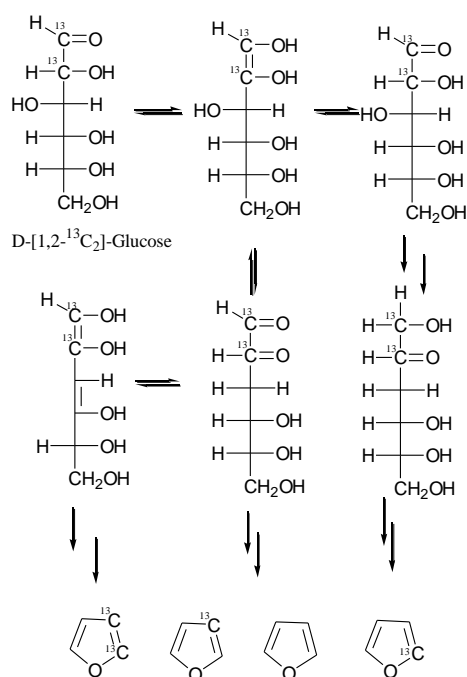


Figure 1.10: Summary schematic of potential mechanistic routes to furan from glucose (72).

The use of labelled sugars has also been applied to the study of specific pathways such as enolisation (89) by analysing where the labelled fragments are located at the end of the reaction. The formation of acetic acid during the Maillard reaction under various conditions has been studied in this way (89) as it is a common product formed by the reaction of hexose sugars in alkaline conditions. The amount of acetic acid formation was studied to establish the effect of pH, temperature and reaction time for a glucose-glycine phosphate buffered system (89). It appears that such studies have not yet been applied in dairy systems (or model systems based on dairy products), but these would provide valuable information about the mechanisms of formation of flavour in dairy food.

There are various dairy products that contain an emulsion structure; cream is an oil in water emulsion, inversely butter is a water in oil emulsion, and processed cheeses can be formed using emulsions (90). Different emulsion or microemulsion structures for a reaction system can also be modelled. These studies have been used to demonstrate the different outcomes of the reaction that could be achieved by changing the structure of dairy foods. Structured fluids were investigated (19) as microreactors for Maillard reaction chemistry. Reactions were carried out in three different systems: an aqueous phase consisting of 100% water; a traditional water-in-oil microemulsion with discrete and continuous phases; and a unique cubic phased system, that had an interesting structure consisting of an interpenetrating network of channels of the two phases, each of which is continuous (19). When the reaction was carried out in the cubic phase, a wider product profile was produced

along with an increase in observed product yields. The transition between microemulsion structures is continuous, allowing bicontinuous microemulsion structures to form (18). These structures can be transitioned between with adjustment of the water content of the system. Since the rate of reaction was altered with changes to the structure, it could therefore be altered by adjusting the amount of water in the system. The same cannot be said for emulsions whose structures are discrete from one another; to transition from o/w to w/o there must be a phase inversion that is transitional rather than catastrophic (91).

With the exception of using novel double emulsions to form processed cheeses (90) there has been little research published on the possibilities of altering the structures of dairy products such as milk, cream and butter or the impact that such structures have on final flavour properties of the products.

1.4.3 Analysis of flavours

The analysis of the products formed is an important aspect of the model studies and studies involving real food systems. A range of techniques are available to study the end products of the reactions depending on the questions being asked: chemical analysis (92-96) is used to identify the reaction compounds that form during the course of the reaction, physical analysis has been used to study the texture, rheology (97) and colour, while sensory analysis (42, 98-100) is used to determine the flavour and texture along with the acceptability of these parameters by the consumer. Most studies focus on one form of analysis, either identifying what the reaction products are, or how the colour changed over the course of the reaction, or whether the consumer finds the product acceptable. To gain a full understanding of dairy product flavour more than one set of analysis is required.

1.5 Flavours in dairy products

In addition to the mild dairy flavours derived from the milk itself, specific dairy products will have characteristic flavours derived from their method of manufacture; the method may influence either the pathways of the Maillard reaction, or the subsequent perception of any Maillard reaction products. The heating processes used in dairy food processing are generally for pasteurisation or water removal. This application of heat initiates the Maillard reaction, which generates additional flavour compounds, resulting either in flavours being produced or in the generation of flavour precursor compounds that go on to react during subsequent cooking. In either scenario, these compounds may be off-flavours or beneficial to product quality.

1.5.1 Fluid milks

In milk, there are over 200 volatile components that contribute to the overall flavour (43). Several of these components are present in very small amounts (<400 µg/kg) but still impact the overall flavour profile. Maillard reaction products have been identified in heat damaged milk (101, 102) as a result of pasteurisation. The presence of these products even at small levels can alter the flavour of the milk producing an off-flavour. There have been many analytical studies focusing on identifying the flavour compounds of milk (28, 103).

UHT milk is heat treated at ultra-high temperatures (54). This brief but intense heating occurs in a temperature range of 135-140 °C. The heating can be either direct (steam injection or milk infusion into steam) or indirect (heating and cooling using heat exchangers). This heat treatment destroys pathogens, but can also cause chemical changes in the milk leading to off-flavours. Ideally, UHT milk would have the same flavour as fresh milk: namely only a minimal aroma, with a slightly sweet but relatively bland flavour. This flavour profile can be greatly influenced by the presence of volatile compounds, in particular sulfur containing compounds. While adding desirable flavour to cheeses and butter, these sulfur compounds, including hydrogen sulphide, dimethyl sulphide and methanethiol, which can all be derived from Maillard chemistry, are often responsible for the off-flavours in UHT milk (53).

1.5.2 Milk powders

Whilst most Maillard reactions are initiated by heat, it has also been demonstrated that Maillard chemistry is an important factor in the development of off-flavours and browning in milk powders during storage (28, 64, 103). These reactions also result in nutritive damage to the product (104) and therefore the conditions of storage are important to minimise these deleterious effects. Any off-flavours, such as nitrogen containing indole compounds, are very noticeable and objectionable in the final product (28, 103).

Likewise, the temperature of milk powder storage can influence the Maillard reaction; samples stored at a higher temperature (50 °C) exhibit accelerated Maillard reaction product formation (104). Increased humidity has also been observed to accelerate the Maillard reaction during storage, if the a_w was increased into the range appropriate for this chemistry to become significant (30). The formation of compounds such as HMF (figure 1.4) and furosine along with browning of the powders are indicators of the Maillard reaction during storage (105, 106). HMF is commonly used as a

Maillard indicator in dairy products, but is not suitable for all food types. The monitoring of HMF is often done using colorimetric techniques (105, 106) or fluorescence (104). An enzyme linked immunoabsorbant assay is currently being developed to monitor HMF in a wide range of carbohydrate containing foods (107).

The furan derivatives furfural and furfuryl alcohol (figure 1.4) are Maillard reaction products produced by sugar degradation and dehydration. These compounds were identified in skim milk powder, but their levels were below the perception threshold. However, they have a sweet, nutty, caramel odour that could be important for condensed milk (28). The Maillard reaction also leads to the development of colour in sweetened condensed milk (106, 108).

1.5.3 Cheese

The flavour of cheese is derived from the activities and interactions of starter bacteria, rennet, milk enzymes and also from any secondary flora present (109). The flavour compounds of impact (64) include methyl ketones formed *via* fat oxidation that is initiated by bacteria, fatty acids formed from the lipolysis of the milk fat, sulfur compounds generated by bacteria, α -dicarbonyl and related compounds that can also react with amino acids to generate Strecker aldehydes (a known Maillard reaction pathway (21)) and amine compounds (64), e.g., the amino termini of peptides generated by enzymatic hydrolysis, which may also participate in Maillard chemistry.

The use of cheese on pizza and in prepared foods requires a cheese with desirable attributes, including colour, after cooking (110). The browning of cheese during baking can be attributed to the Maillard reaction. When tested by sensory panellists, the cooked brown colour and flavour of the cooked cheese were not seen as undesirable, although a less coloured cooked cheese was favoured by pizza manufacturers surveyed (111).

The manufacture of processed cheese involves heating the cheese products with other materials (112) to yield a homogeneous product. The temperature of the processing steps can cause colour defects (browning) that have been linked to the Maillard reaction. Aroma defects are also generated giving rise to an 'overcooked' aroma. These defects depend on the heating time and temperature and the product composition (112). There were several compounds identified in a model cheese system that are known Maillard reaction products: furfuryl alcohol, furfural, furaneol and maltol (112). This model system demonstrated that these compounds played a major role in the 'overcooked' defect (112).

1.5.4 Flavours in cooked dairy products

The heating of dairy products is not limited to manufacturing, as it is also a common household process (113). Flavour is an important attribute of food, both prior to and after heating. Many dairy products are cooked, such as cheeses, cream and butter. There are also products that are made by cooking other dairy ingredients, including ghee, milk chocolate crumb, Ryazhenka kefir (10) and browned butter. The effects of the Maillard reaction are often seen when these products are cooked, providing a desired change to the product, including grilled cheese such as on pizza, browned butter often used in making roux or baking, milk chocolate crumb or block milk (12, 13).

Halloumi is a cheese that is eaten after frying or grilling, developing a browned outer layer. Although little sensory analysis has been carried out on this cheese, it is known that the volatile flavour compounds differ between the raw and cooked cheese (98). Moreover, given that this cheese is most commonly consumed in cooked form, the change in flavour can be assumed to be desirable. Another cheese commonly cooked for consumption is mozzarella; there were no undesirable flavours noted when mozzarella was cooked (111).

Cream, milk and butter are also commonly used in baked goods such as scones, shortbreads and other sweet items, where they are subjected to high temperatures, in the presence of sugar, for cooking; many Maillard reaction products are thus generated that are associated with the favourable aroma of these products. A further use of the Maillard reaction in cooking is during the making of ghee. Ghee is an example of a product in which the flavour compounds generated *via* the Maillard reaction are desirable. The flavour in ghee is generated from multiple sources: compounds found naturally in the starting material, Maillard browning reaction compounds, free fatty acids, lipid oxidation and fermentation (9). Dulce de leche is another product that requires the Maillard reaction to generate the characteristic colour and flavour. The consumer acceptability relies on the flavour and colour (99).

1.5.5 Low fat dairy products

The current consumer driven market is searching for low fat food alternatives, including dairy products, without compromising on food flavour; however, low fat versions of products such as cheeses, milk, ice cream and yoghurts often lack flavour. Fat can be replaced with protein, carbohydrates, other fat based products, or a combination of all three (100, 114). Low fat cheese can lose up to 50% of its flavour with a reduction in fat (81), which, coupled with associated changes in texture and other sensory attributes (81, 100), can lead to an inferior product. For example, low fat

chocolate ice-cream made with 2.5% milk fat plus low fat cocoa powder instead of cocoa butter had a less intense flavour (100).

Ghee is a fat product with potential for use in flavour replacement. The highly flavoured nature of ghee relative to products such as butter oil or anhydrous milk fat (AMF) makes it an alternative that would add flavour, even at low fat content.

1.5.6 Ghee

Ghee is a product that is made by indigenous methods (9) in many countries around the world, largely in Asia, the Middle East and Africa. It is known by various other names such as maslee in the Middle East where ghee is derived from goat, sheep or camel milk (9, 29, 115) and roghan in Iran. Ghee is used in cooking, as a condiment and for religious purposes (116). The cooking methods vary along with the unique individual flavours (9).

By definition, ghee is 'a product exclusively obtained from milk, cream or butter by means of processes which result in almost total removal of water and non-fat solids, with an especially developed flavour and physical structure' (117); the flavour is 'acceptable for market requirements after heating a sample to 40-45 °C' (117). A good ghee has been defined (117) as having a pleasant, nutty, lightly cooked or caramelised flavour. It was said that the flavour can best be described as a lack of oiliness or blandness, sweetly rather than sharply acidic (8), but it is objectionable to have any suggestion of rancidity. The flavour of ghee cannot be attributed to a single compound, but rather to a large range of compounds (8). This group of compounds includes aldehydes, ketones, fatty acids, carboxylic acids, lactones and alcohols (29, 116). These flavours are generated during the heating process by reactions between protein and lactose, protein and lactose degradation products, lipid oxidation and degradation of free fatty acids (9).

There are several methods of making indigenous ghee; all have a common element of heating to high temperature (110-140 °C). The different methods of making ghee all start from fermented milk, cream (often soured to 0.5-1% acidity), butter or a combination of butter and buttermilk (9, 118). After the heat clarification, the remaining product is filtered to remove any remaining solids (section 3.2).

The presence of maltol and furans has been linked to significant differences in flavours (116). These products, along with other pyrans, various ketones and aldehydes are known Maillard reaction products (21, 116). Maltol and various furans are products of sugar degradation, and the initial

reducing sugar concentration can greatly influence the amount of each compound formed (116). Important flavour compounds are considered to be aldehydes, ketones and lactones (9, 119).

There are commercial products that fit the definition of ghee based solely on physical characteristics. These products include AMF (anhydrous milk fat), butter ghee and butter oil (120). Whilst they are similar with respect to some of their characteristics, these products do not display the same flavour profile as traditional indigenous ghee; butter oil and AMF are particularly lacking in flavour. The production of synthetic ghee flavours (8, 118) has been attempted in the past using a formulation of synthetic flavouring compounds to create a ghee flavour in butter oil. The synthetic flavourings used were based on analytical data for ghee flavour constituents (8). Efforts made to reconstitute the flavours focussed on a mixture of lactones, free fatty acids and carbonyl compounds (8); however, as not all of the ghee constituents had been identified, and their exact proportions were not known, it was difficult to formulate a synthetic ghee flavour in the butteroil (8).

There are several papers (9, 29, 115, 116, 121) that outline the flavour constituents that have been identified in ghee. The compounds identified are generally grouped into aldehydes, ketone (including methyl ketones), esters, acids, alcohols and lactones with any compounds not fitting these groups identified as 'other'. Within these groups there are several compounds that can be attributed to formation *via* the Maillard reaction. These compounds (figure 1.11) include HMF, acetic acid, maltol (larixinic acid), furfuryl alcohol, furfural, hydroxymaltol (8, 9, 29, 116). A better understanding of high temperature Maillard chemistry may therefore inform the development of this type of product and allow the manipulation of flavour.

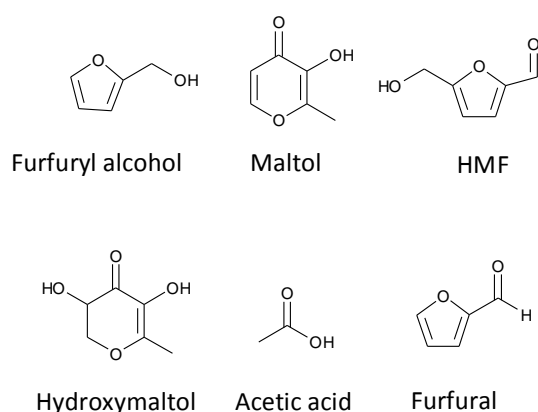


Figure 1.11 Maillard reaction compounds identified in ghee (8, 9, 29, 116).

1.6 Mechanistic pathways

The use of model studies to investigate the pathways of the Maillard and caramelisation reactions has led to the elucidation of mechanisms of formation of a variety of compounds, some of which are found in the dairy products above (such as those depicted in figures 1.4 and 1.11).

The formation of the Amadori compound and its degradation along the 1,2-enolisation and 2,3-enolisation pathways have been studied in detail (figures 1.2 and 1.3) (1, 20, 21, 27) up to the formation of the keto and enol derivatives. However, the elucidation of mechanisms for compound formation after this point has been limited to studies focusing on a single or related group of compounds. The published mechanisms are not always complete, with some containing missing steps where only the intermediates detected through analysis are presented in the mechanisms. There have been a wide range of compounds studied, of which only some are found in dairy products.

Important flavour compounds for dairy products, particularly those with caramel flavours, are maltol and hydroxymaltol (figures 1.12 and 1.13). These two compounds can be formed from the 2,3-enolisation pathway of the Maillard reaction cascade and, in turn, have been shown to degrade further under certain conditions (10, 57, 122-126).

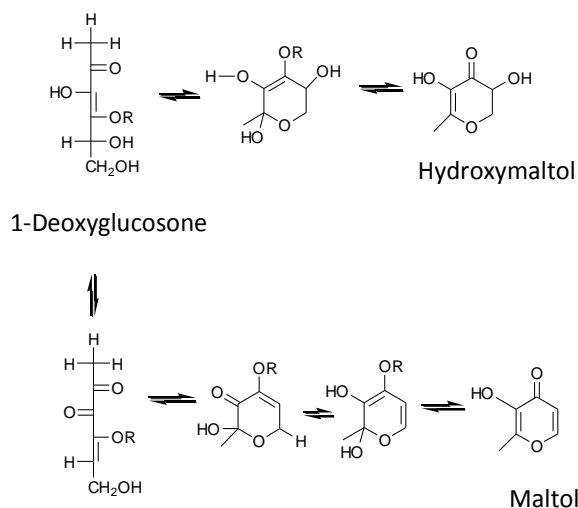


Figure 1.12 Formation of hydroxymaltol and maltol from the 1-deoxyosone intermediate formed along the 2,3-enolisation pathway (57, 126).

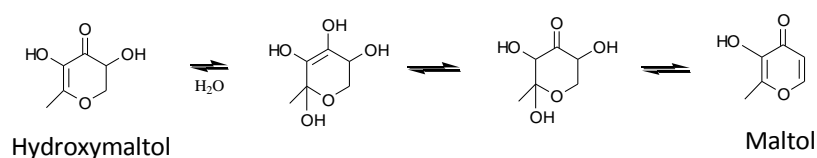


Figure 1.13 Degradation of hydroxymaltol (1) to maltol (2) as described by Bates and Kim (122).

Along the 2,3-enolisation pathway acetic acid is also formed with several hypothesised mechanisms (89, 127), from the 1-deoxy-2,3-hexodiulose structure. The mechanisms were determined for reactions using glucose as the sugar rather than lactose. The C4 fragment formed (figure 1.14) can then react further to generate C4 furanone compounds (21).

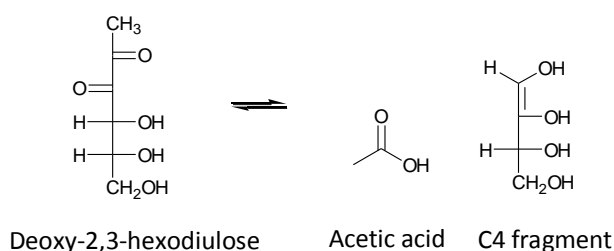


Figure 1.14 Formation of acetic acid and C4 reactive fragment (89, 127).

The deoxy-2,3-hexodiulose compound also provides a pathway to 2-acetylfuran (31) *via* the 1,4-dideoxyosone compound as shown in figure 1.15. The 2-acetylfuran was formed from glucose and glycine but could also be formed from glucose *via* a similar pathway from the 1,4-dideoxyosone compound.

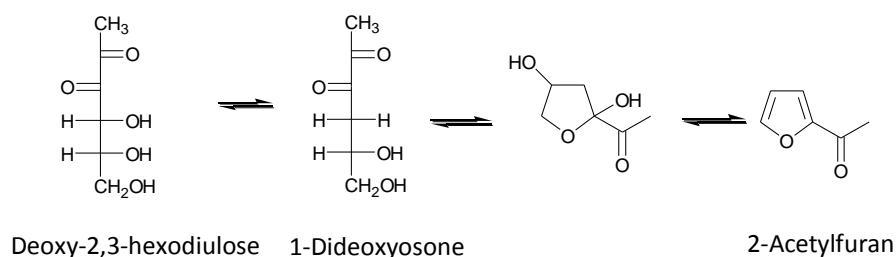


Figure 1.15 Formation of 2-acetylfuran *via* 2,3-enolisation pathway (31).

The Amadori compound can also degrade *via* a 1,2-enolisation mechanism (figure 1.3) to form furfural, furfuryl alcohol and HMF (21, 57). The 3-deoxyhexosone (figure 1.16) is formed *via* the 1,2-enolisation pathway en route to the formation of HMF.

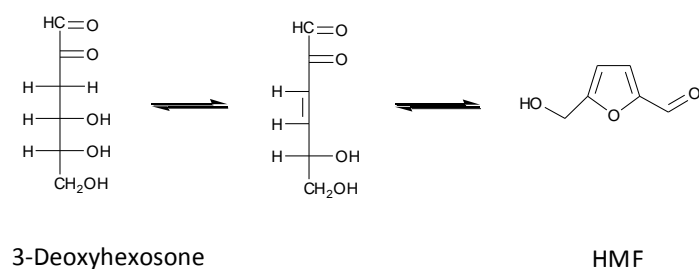


Figure 1.16 Formation of HMF (57).

The formation of furfuryl alcohol (figure 1.17) can take place through a deoxy-pentose compound (38, 128) that forms *via* a retro-Claisen mechanism from the 1-amino-1,4-dideoxyhexosulose compound to a pentose intermediate compound. The pentose intermediate is formed along the 1,2-enolisation pathway from the Amadori compound.

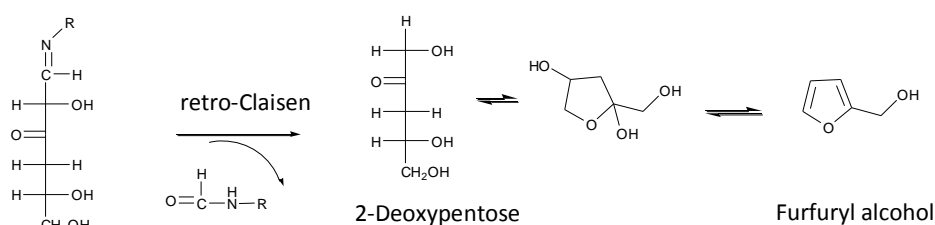


Figure 1.17 Furfuryl alcohol formation *via* 2-deoxypentose compound formed by a retro-Claisen reaction of an aminoreductone (128, 129).

Each of the reaction pathways have been studied using various sugar and amino acid combinations. These mechanisms can be used in to form a hypothetical map of pathways that explains the formation of volatile compounds in dairy products *via* the Maillard and caramelisation reactions and may help guide experiments to manipulate their relative concentrations. Some postulated mechanisms are described in Appendix A.

1.7 Conclusion

In the majority of dairy products the Maillard reaction is often an undesirable source of flavours, which has led to a focus by the dairy industry on reducing the extent of the reaction. However, in cooked cream products, such as ghee and dulce de leche, Maillard reactions are desirable and indeed essential.

Some dairy foods actually have very little in common with the model systems that have been used to study the Maillard reaction. Although the use of models to study the influence of microemulsion structures on the Maillard reaction has demonstrated that different microemulsion structures can

lead to different reaction product profiles, many food systems are emulsions (cream and butter) rather than microemulsions, leaving a significant gap between models and food, which needs to be bridged.

Many of the flavour studies have been conducted with the purpose of identifying marker compounds found in the products. There has been a lack of studies that systematically identify the flavour compounds in dairy products and link the formation of these compounds to the food system variables such as emulsion structure. Information from such studies could be used to control compound formation and so control the flavour profile.

Numerous flavour compounds have been found in ghee, but little work has been done to determine the origin of these compounds, the mechanisms by which they form, and the influence of the emulsion structure on the final product ratios. More work is required to understand the mechanisms of flavour formation in cooked cream products, in particular, so that the flavour can be manipulated to enhance it and make the final product more desirable.

1.8 Thesis aims/Chapter outlines

The aim of this thesis was to determine the influence of emulsion structure in ghee, on the formation of flavour compounds through the Maillard and related reactions. To achieve the aim the following objectives needed to be completed:

1. To develop a SPME/GCMS method for screening of Maillard reaction compounds formed in ghee and model reaction systems.
2. To develop a high throughput method for cooking ghee.
3. To develop a model reaction system and a model matrix system to study the influence of emulsion structure on the Maillard reaction.
4. To determine the influence of different emulsion structures on the Maillard reaction.

Chapter two discusses the development of the SPME/GCMS analysis method.

Chapter three introduces ghee, its traditional manufacture and a high throughput method of cooking.

Chapter four presents the experimental model reactions for the caramelisation reaction and the Maillard reactions along with a study of the influence of pH, salts and buffers.

Chapter five discusses the addition of fat to the reaction matrix, describes the influence of fat on the reaction models and looks at the impact of different types of fat.

Chapter six presents the development of different emulsion structures and an assessment of their influence on the Maillard and caramelisation reaction models.

Chapter seven provides the final conclusions and important findings of the thesis.

Chapter eight discusses the materials and methods used throughout the thesis.

Appendix A includes plausible reaction mechanisms by which the compounds monitored during the experiments may be formed.

Appendix B includes Fonterra Cooperative Group product bulletins.

Appendix C provides information regarding statistical methods and statistical data.

1.9 References

1. Nursten, H. E., The flavour of milk and dairy products. 1. Milk of different kinds, milk powder, butter and cream. *International Journal of Dairy Technology* **1997**, *50*, 48-56.
2. Ames, J. M., Applications of the Maillard reaction in the food industry. *Food Chemistry* **1998**, *62*, 431-439.
3. van Boekel, M. A. J. S., Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances* **2006**, *24*, 230-233.
4. Keramat, J.; LeBail, A.; Prost, C.; Soltanizadeh, N., Acrylamide in foods: chemistry and analysis. A review. *Food and Bioprocess Technology* **2011**, *4*, 340-363.
5. Van Nguyen, C., Toxicity of the AGEs generated from the Maillard reaction: On the relationship of food-AGEs and biological-AGEs. *Molecular Nutrition and Food Research* **2006**, *50*, 1140-1149.
6. van Boekel, M. A. J. S., Effect of heating on Maillard reactions in milk. *Food Chemistry* **1998**, *62*, 403-414.
7. Hiller, B.; Lorenzen, P. C., Functional properties of milk proteins as affected by Maillard reaction induced oligomerisation. *Food Research International* **2010**, *43*, 1155-1166.
8. Yadav, J. S.; Srinivasan, R. A., Advances in ghee flavour research. *Indian Journal of Dairy Science* **1992**, *45*, 338-348.
9. Sserunjogi, M. L.; Abrahamsen, R. K.; Narvhus, J., A review paper: Current knowledge of ghee and related products. *International Dairy Journal* **1998**, *8*, 677-688.
10. Preininger, M.; Gimelfarb, L.; Li, H. C.; Dias, B. E.; Fahmy, F.; White, J., Identification of dihydromaltol (2,3-dihydro-5-hydroxy-6-methyl-4(H)-pyran-4-one) in Ryazhenka kefir and comparative sensory impact assessment of related cycloenolones. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 9902-9908.
11. Pauletti, M. S.; Matta, E. J.; Rozycki, S., Kinetics of heat-induced browning in concentrated milk with sucrose as affected by pH and temperature. *Food Science and Technology International* **1999**, *5*, 407-413.
12. Muresan, S.; Eillebrecht, M.; de Rijk, T. C.; de Jonge, H. G.; Leguijt, T.; Nijhuis, H. H., Aroma profile development of intermediate chocolate products I. Volatile constituents of block-milk. *Food Chemistry* **2000**, *68*, 167-174.
13. Edmondson, P. T.; Grammatika, M.; Fryer, P. J.; Handy, B., Modelling of heat transfer, mass transfer and flavour development in chocolate crumb. *Food and Bioprocess Technology* **2005**, *83*, 89-98.
14. Fanun, M.; Leser, M.; Aserin, A.; Garti, N., Sucrose ester microemulsions as microreactors for model Maillard reaction. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2001**, *194*, 175-187.
15. Garti, N., Microemulsions as microreactors for food applications. *Current Opinion in Colloid and Interface Science* **2003**, *8*, 197-211.

16. Yaghmur, A.; Fanun, M.; Aserin, A.; Garti, N., Food grade microemulsions based on nonionic emulsifiers as selective microreactors for flavor formation by Maillard reaction. *Self-Assembly* **2003**, 144-151.
17. Yaghmur, A.; Aserin, A.; Abbas, A.; Garti, N., Reactivity of furfural-cysteine model reaction in food-grade five-component nonionic o/w microemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2005**, 253, 223-234.
18. Holmberg, K., Organic reactions in microemulsions. *European Journal of Organic Chemistry* **2007**, 731-742.
19. Vauthey, S.; Milo, C.; Frossard, P.; Garti, N.; Leser, M. E.; Watzke, H. J., Structured fluids as microreactors for flavor formation by the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2000**, 48, 4808-4816.
20. Ledl, F.; Schleicher, E., New aspects of the Maillard reaction in foods and in the human body. *Angewandte Chemie - International Edition* **1990**, 29, 565-594.
21. Nursten, H. E., *The Maillard reaction: chemistry, biochemistry and implications*. RSC Publishing: Cambridge, UK, 2005.
22. Rizzi, G. P., The Strecker degradation of amino acids: Newer avenues for flavor formation. *Food Reviews International* **2008**, 24, 416-435.
23. Whitfield, F. B., Volatiles from interactions of Maillard reactions and lipids. *Critical Reviews in Food Science and Nutrition* **1992**, 31, 1-58.
24. Hidalgo, F. J.; Nogales, F.; Zamora, R., The role of amino phospholipids in the removal of the cito- and geno-toxic aldehydes produced during lipid oxidation. *Food and Chemical Toxicology* **2008**, 46, 43-48.
25. Metwalli, A. A. M.; van Boekel, M., On the kinetics of heat-induced deamidation and breakdown of caseinate. *Food Chemistry* **1998**, 61, 53-61.
26. Fayle, S. E.; Gerrard, J. A., *The Maillard reaction*. Royal Society of Chemistry: Cambridge, UK, 2002.
27. Gerrard, J. A., New aspects of an AGEing Chemistry - Recent developments concerning the Maillard reaction. *Australian Journal of Chemistry* **2002**, 55, 299-310.
28. Shiratsuchi, H.; Shimoda, M.; Imayoshi, K.; Noda, K.; Osajima, Y., Volatile flavour compounds in spray-dried skim milk powder. *Journal of Agricultural and Food Chemistry* **1994**, 42, 984-988.
29. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C.; Przybylski, R., Evaluation of volatile compounds in different types of ghee using headspace with GC/MS. *Milchwissenschaft - Milk Science International* **2003**, 58, 37-39.
30. Le, T. T.; Bhandari, B.; Deeth, H. C., Chemical and physical changes in milk protein concentrate (MPC80) powder during storage. *Journal of Agricultural and Food Chemistry* **2011**, 59, 5465-5473.

31. Wang, Y.; Juliani, H. R.; Simon, J. E.; Ho, C. T., Amino acid-dependent formation pathways of 2-acetylfuran and 2,5-dimethyl-4-hydroxy-3(2H)-furanone in the Maillard reaction. *Food Chemistry* **2009**, *115*, 233-237.
32. van Boekel, M., Kinetic aspects of the Maillard reaction: a critical review. *Nahrung-Food* **2001**, *45*, 150-159.
33. Brands, C. M. J.; van Boekel, M., Reactions of monosaccharides during heating of sugar-casein systems: Building of a reaction network model. *Journal of Agricultural and Food Chemistry* **2001**, *49*, 4667-4675.
34. van Boekel, M. A. J. S.; Brands, C. M. J., Heating of sugar-casein solutions: Isomerization and Maillard reactions. In *The Maillard reaction in foods and medicine*, O'Brien, J.; Nursten, H. E.; Crabbe, M. J.; Ames, J. M., Eds. Woodhead publishing: Cambridge, UK, 1998; pp 154-159.
35. Monti, S. M.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A. L.; Fogliano, V., LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *Journal of Agricultural and Food Chemistry* **1999**, *47*, 1506-1513.
36. Yu, A. N.; Zhang, A. D., The effect of pH on the formation of aroma compounds produced by heating a model system containing L-ascorbic acid with L-threonine/L-serine. *Food Chemistry* **2010**, *119*, 214-219.
37. Limacher, A.; Kerler, J.; Conde-Petit, B.; Blank, I., Formation of furan and methylfuran from ascorbic acid in model systems and food. *Food Additives and Contaminants* **2007**, *24*, 122-135.
38. Berg, H. E.; Vanboekel, M., Degradation of lactose during heating of milk. 1. Reaction pathways. *Netherlands Milk and Dairy Journal* **1994**, *48*, 157-175.
39. Tomasik, P.; Palasinski, M.; Wiejak, S., The thermal decomposition of carbohydrates. 1. The decomposition of monosaccharides, disaccharides and oligosaccharides. *Advances in Carbohydrate Chemistry and Biochemistry* **1989**, *47*, 203-278.
40. Wrolstad, R. E., Reactions of sugars. In *Food carbohydrate chemistry*, 1 ed.; Wrolstad, R. E., Ed. John Wiley and Sons Inc: 2012; pp 35 - 47.
41. Aider, M.; de Halleux, D., Isomerization of lactose and lactulose production: review. *Trends in Food Science and Technology* **2007**, *18*, 356-364.
42. Wong, K. H.; Aziz, S. A.; Mohamed, S., Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. *International Journal of Food Science and Technology* **2008**, *43*, 1512-1519.
43. McSweeney, P. L. H.; Nursten, H. E.; Urbach, G., *Advanced dairy chemistry vol 3: Lactose, water, salts and vitamins*. 2 ed.; Chapman and Hall: London, UK, 1997; Vol. 3.
44. Hajek, J.; Murzin, D. Y.; Salmi, T.; Mikkola, J. P., Interconversion of lactose to lactulose in alkaline environment: comparison of different catalysis concepts. *Topics in Catalysis* **2013**, *56*, 839-845.
45. Ganzle, M. G.; Haase, G.; Jelen, P., Lactose: Crystallization, hydrolysis and value-added derivatives. *International Dairy Journal* **2008**, *18*, 685-694.

46. Hellwig, M.; Degen, J.; Henle, T., 3-Deoxygalactosone, a "new" 1,2-dicarbonyl compound in milk products. *Journal of Agricultural and Food Chemistry* **2010**, *58*, 10752-10760.
47. McMurry, J., *Organic chemistry*. 5 ed.; Brooks/Cole Thomson Learning: Pacific Grove, CA, United States of America, 1999.
48. Guan, Y. G.; Wang, S. L.; Yu, S. J.; Yu, S. M.; Zhao, Z. G., Changes in the initial stages of a glucose-proline Maillard reaction model system influences dairy product quality during thermal processing. *Journal of Dairy Science* **2012**, *95*, 590-601.
49. Bell, L. N., Maillard reaction as influenced by buffer type and concentration. *Food Chemistry* **1997**, *59*, 143-147.
50. Yaylayan, V. A.; Machiels, D.; Istasse, L., Thermal decomposition of specifically phosphorylated D-glucoses and their role in the control of the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 3358-3366.
51. Yaghmur, A.; Aserin, A.; Garti, N., Furfural-cysteine model reaction in food grade nonionic oil/water microemulsions for selective flavor formation. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 2878-2883.
52. Ajandouz, E. H.; Desseaux, V.; Tazi, S.; Puigserver, A., Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry* **2008**, *107*, 1244-1252.
53. Al-Attabi, Z.; D'Arcy, B. R.; Deeth, H. C., Volatile sulphur compounds in UHT milk. *Critical Reviews in Food Science and Nutrition* **2009**, *49*, 28-47.
54. TetraPak, *Dairy processing handbook*. Tetra Pak Processing Systems: Lund, Sweden, 1995.
55. Lioumbas, J. S.; Karapantsios, T. D., Evaporation front compared with crust thickness in potato deep-fat frying. *Journal of Food Science* **2011**, E17-E25.
56. Voziyan, P. A.; Metz, T. O.; Baynes, J. W.; Hudson, B. G., A post-Amadori inhibitor pyridoxamine also inhibits chemical modification of proteins by scavenging carbonyl intermediates of carbohydrate and lipid degradation. *Journal of Biological Chemistry* **2002**, *277*, 3397-3403.
57. Parker, J. K., The kinetics of thermal generation of flavour. *Journal of the Science of Food and Agriculture* **2013**, *93*, 197-208.
58. Adams, A.; Kitryte, V.; Venskutonis, R.; De Kimpe, N., Model studies on the pattern of volatiles generated in mixtures of amino acids, lipid-oxidation-derived aldehydes, and glucose. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 1449-1456.
59. Ho, C. T.; Chen, Q. Y., Lipids in food flavours - An overview. In *Lipids in food flavors*, Ho, C. T.; Hartman, T. G., Eds. American Chemical Society: Washington, DC, United States of America, 1994; Vol. 558, pp 2-14.
60. Singh, S.; Ram, B. P.; Mittal, S. K., Effect of phospholipids and method of manufacture on flavour and keeping quality of ghee. *Indian Journal of Dairy Science* **1979**, *32*.
61. Giroux, H. J.; Houde, J.; Britten, M., Use of heated milk protein-sugar blends as antioxidant in dairy beverages enriched with linseed oil. *LWT - Food Science and Technology* **2010**, *43*, 1373-1378.

62. Lutz, R.; Aserin, A.; Garti, N., Maillard reaction between leucine and glucose in o/w microemulsion media in comparison to aqueous solution. *Journal of Dispersion Science and Technology* **2005**, *26*, 535-547.
63. Malec, L. S.; Gonzales, A. S. P.; Naranjo, G. B.; Vigo, M. S., Influence of water activity and storage temperature on lysine availability of a milk like system. *Food Research International* **2002**, *35*, 849-853.
64. Gonzales, A. S. P.; Naranjo, G. B.; Leiva, G. E.; Malec, L. S., Maillard reaction kinetics in milk powder: Effect of water activity at mild temperatures. *International Dairy Journal* **2010**, *20*, 40-45.
65. Fernandez-Salguero J., G. R., Carmona M. A., Water activity of selected high-moisture foods. *Journal of Food Composition and Analysis* **1993**, *6*, 364-369.
66. Shrestha, A. K.; Howes, T.; Adhikari, B. P.; Bhandari, B. R., Spray drying of skim milk mixed with milk permeate: Effect on drying behavior, physicochemical properties, and storage stability of powder. *Drying Technology* **2008**, *26*, 239-247.
67. Lloyd, M. A.; Drake, M. A.; Gerard, P. D., Flavor variability and flavor stability of US-produced whole milk powder. *Journal of Food Science* **2009**, *74*, S334-S343.
68. Rizzi, G. P., Effects of cationic species on visual color formation in model Maillard reactions of pentose sugars and amino acids. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 7160-7164.
69. Morgan, J.; Leake, D. S., Oxidation of low density lipoprotein by iron or copper at acidic pH. *Journal of Lipid Research* **1995**, *36*, 2504-2512.
70. Moulik, S. P.; Paul, B. K., Structure, dynamics and transport properties of microemulsions. *Advances in Colloid and Interface Science* **1998**, *78*, 99-195.
71. van Boekel, M. A. J. S., Predictive modeling of flavour compound formation in the Maillard reaction: A SWOT analysis. In *In controlling Maillard pathways to generate flavors*, Oxford University Press: Cary, NC, United States of America, 2010; Vol. 1042, pp 1-11.
72. Limacher, A.; Kerler, J.; Davidek, T.; Schmalzried, F., Formation of furan and methylfuran by Maillard-type reaction in model systems and food. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 3639-3647.
73. Illmann, S.; Davidek, T.; Gouezec, E.; Rytz, A.; Schuchmann, H. P.; Blank, I., Generation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone from rhamnose as affected by reaction parameters: experimental design approach. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 2889-2895.
74. Engel, W.; Hofmann, T.; Schieberle, P., Characterization of 3,4-dihydroxy-3-hexen-2,5-dione as the first open-chain caramel-like smelling flavor compound. *European Food Research and Technology* **2001**, *213*, 104-106.
75. Wijewickreme, A. N.; Kitts, D. D., Influence of reaction conditions on the oxidative behavior of model Maillard-reaction products. *Journal of Agricultural and Food Chemistry* **1997**, *45*, 4571-4576.
76. Yoshimura, Y.; Iijima, T.; Watanabe, T.; Nakazawa, H., Antioxidative effect of Maillard reaction products using glucose-glycine model system. *Journal of Agricultural and Food Chemistry* **1997**, *45*, 4106-4109.

77. Akhtar, M.; Murray, B. S.; Dickinson, E., Perception of creaminess of model oil-in-water dairy emulsions: Influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. *Food Hydrocolloids* **2006**, *20*, 839-847.
78. Brands, C. M. J.; van Boekel, M., Kinetic modelling of reactions in heated disaccharide-casein systems. *Food Chemistry* **2003**, *83*, 13-26.
79. Rozycki, S. D.; Buera, M. P.; Pauletti, M. S., Heat-induced changes in dairy products containing sucrose. *Food Chemistry* **2010**, *118*, 67-73.
80. Hernandez, H. G.; Livings, S.; Aguilera, J. M.; Chiralt, A., Phase transitions of dairy proteins, dextrans and their mixtures as a function of water interactions. *Food Hydrocolloids* **2011**, *25*, 1311-1318.
81. Drake, M. A.; Miracle, R. E.; McMahon, D. J., Influence of fat on flavour and flavour development in cheddar cheese. *Australian Journal of Dairy Technology* **2010**, *65*, 195-199.
82. Druaux, C.; Voilley, A., Effect of food composition and microstructure on volatile flavour release. *Trends in Food Science and Technology* **1997**, *8*, 364-368.
83. Hofmann, T.; Schieberle, P.; Grosch, W., Model studies on the oxidative stability of odor-active thiols occurring in food flavors. *Journal of Agricultural and Food Chemistry* **1996**, *44*, 251-255.
84. Philippe, E.; Seuvre, A. M.; Colas, B.; Langendorff, V.; Schippa, C.; Voilley, A., Behavior of flavor compounds in model food systems: a thermodynamic study. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 1393-1398.
85. Morales, F. J.; Jimenez-Perez, S., HMF formation during heat-treatment of milk-type products as related to milkfat content. *Journal of Food Science* **1999**, *64*, 855-859.
86. Ajandouz, E. H.; Tchiakpe, L. S.; Dalle Ore, F.; Benajiba, A.; Puigserver, A., Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science* **2001**, *66*, 926-931.
87. Hwang, I. G.; Kim, H. Y.; Woo, K. S.; Lee, J.; Jeong, H. S., Biological activities of Maillard reaction products (MRPs) in a sugar-amino acid model system. *Food Chemistry* **2011**, *126*, 221-227.
88. Wang, H. Y.; Qian, H.; Yao, W. R., Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry* **2011**, *128*, 573-584.
89. Davidek, T.; Devaud, S.; Robert, F.; Blank, I., Sugar fragmentation in the Maillard reaction cascade: Isotope labeling studies on the formation of acetic acid by a hydrolytic beta-dicarbonyl cleavage mechanism. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 6667-6676.
90. Lobato-Calleros, C.; Sosa-Perez, A.; Rodriguez-Tafoya, J.; Sandoval-Castilla, O.; Perez-Alonso, C.; Vemon-Carter, E. J., Structural and textural characteristics of reduced-fat cheese-like products made from W₁/O/W₂ emulsions and skim milk. *LWT - Food Science and Technology* **2008**, *41*, 1847-1856.
91. Liu, Y. H.; Friberg, S. E., Perspectives of phase changes and reversibility on a case of emulsion inversion. *Langmuir* **2010**, *26*, 15786-15793.

92. Fay, L. B.; Brevard, H., Contribution of mass spectrometry to the study of the Maillard reaction in food. *Mass Spectrometry Reviews* **2005**, *24*, 487-507.
93. Vallejo-Cordoba, B.; Gonzalez-Cordova, A. F., CE: A useful analytical tool for the characterization of Maillard reaction products in foods. *Electrophoresis* **2007**, *28*, 4063-4071.
94. Porretta, S., Chromatographic analysis of Maillard reaction products. *Journal of Chromatography* **1992**, *624*, 211-219.
95. Mariaca, R.; Bosset, J. O., Instrumental analysis of volatile (flavour) compounds in milk and dairy products. *Lait* **1997**, *77*, 13-40.
96. Andersen, C. M.; Mortensen, G., Fluorescence spectroscopy: A rapid tool for analyzing dairy products. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 720-729.
97. Ranalli, N.; Andres, S. C.; Califano, A. N., Physicochemical and rheological characterization of 'dulce de leche'. *Journal of Texture Studies* **2012**, *43*, 115-123.
98. Piggott, J. R.; Margomenou, A.; Withers, S. J.; Conner, J. M., Sensory characterization of Halloumi cheese and relationship with headspace composition. In *Food flavors: Formation, analysis, and packaging influences*, 1st ed.; Contis, E. T.; Ho, C. T.; Mussinan, C. J.; Parliment, T. H.; Shahidi, F.; Spanier, A. M., Eds. Elsevier Science: Philadelphia, PA, United States of America, 1998; Vol. 40, pp 385-391.
99. Gimenez, A.; Ares, G.; Gambaro, A., Consumer reaction to changes in sensory profile of dulce de leche due to lactose hydrolysis. *International Dairy Journal* **2008**, *18*, 951-955.
100. Prindiville, E. A.; Marshall, R. T.; Heymann, H., Effect of milk fat, cocoa butter, and whey protein fat replacers on the sensory properties of lowfat and nonfat chocolate ice cream. *Journal of Dairy Science* **2000**, *83*, 2216-2223.
101. Erbersdobler, H. F.; Somoza, V., Forty years of furosine - Forty years of using Maillard reaction products as indicators of the nutritional quality of foods. *Molecular Nutrition & Food Research* **2007**, *51*, 423-430.
102. Morales, F. J.; Romero, C.; Jimenez-Perez, S., Characterization of industrial processed milk by analysis of heat-induced changes. *International Journal of Food Science and Technology* **2000**, *35*, 193-200.
103. Shiratsuchi, H.; Shimoda, M.; Imayoshi, K.; Noda, K.; Osajima, Y., Off-flavour compounds in spray-dried skim milk powder. *Journal of Agricultural and Food Chemistry* **1994**, *42*, 1323-1327.
104. Liu, X. M.; Metzger, L. E., Application of fluorescence spectroscopy for monitoring changes in nonfat dry milk during storage. *Journal of Dairy Science* **2007**, *90*, 24-37.
105. Oral, R. A.; Dogan, M.; Sarioglu, K., Monitoring of the hydroxymethylfurfural amount as an indicator of Maillard products in sweet whey and skim milk powder stored under different conditions. *Milchwissenschaft - Milk Science International* **2011**, *66*, 398-401.
106. Patel, A. A.; Gandhi, H.; Singh, S.; Patil, G. R., Shelf-life modeling of sweetened condensed milk based on kinetics of Maillard browning. *Journal of Food Processing and Preservation* **1996**, *20*, 431-451.

107. Fang, G. Z.; Lv, Y. Y.; Sheng, W.; Liu, B.; Wang, X. X.; Wang, S., Development of an enzyme-linked immunosorbent assay for the determination of 5-hydroxymethyl-2-furfural in food. *Analytical and Bioanalytical Chemistry* **2011**, *401*, 3367-3373.
108. Kass, J. P.; Palmer, L. S., Browning of autoclaved milk - Chemical factors involved. *Industrial and Engineering Chemistry* **1940**, *32*, 1360-1366.
109. Urbach, G., The flavour of milk and dairy products. 2. Cheese: contribution of volatile compounds. *International Journal of Dairy Technology* **1997**, *50*, 79-89.
110. Wang, H. H.; Sun, D. W., Assessment of cheese browning affected by baking conditions using computer vision. *Journal of Food Engineering* **2003**, *56*, 339-345.
111. Matzdorf, B.; Cuppett, S. L.; Keeler, L.; Hutkins, R. W., Browning of mozzarella-cheese during high-temperature pizza baking. *Journal of Dairy Science* **1994**, *77*, 2850-2853.
112. Bertrand, E.; Machado-Maturana, E.; Chevarin, C.; Portanguen, S.; Mercier, F.; Tournayre, P.; Abouelkaram, S.; Guillard, A. S.; Kondjoyan, A.; Berdague, J. L., Heat-induced volatiles and odour-active compounds in a model cheese. *International Dairy Journal* **2011**, *21*, 806-814.
113. van Boekel, M. A. J. S.; Fogliano, V.; Pellegrini, N.; Stanton, C.; Scholz, G.; Lalljie, S.; Somoza, V.; Knorr, D.; Jasti, P. R.; Eisenbrand, G., A review on the beneficial aspects of food processing. *Molecular Nutrition and Food Research* **2010**, *54*, 1215-1247.
114. Nasirian, R.; Vaziri, A.; Safekordi, A. A.; Ardjmand, M., Use of gum tragacanth in reduction of dairy cream fat. *Milchwissenschaft - Milk Science International* **2010**, *65*, 49-52.
115. Sawaya, W. N.; Khan, P.; Alshalhat, A. F., Physical and chemical characteristics of ghee and butter from goats and sheeps milk. *Food Chemistry* **1984**, *14*, 227-232.
116. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C., Evaluation of volatile compounds in different types of ghee using direct injection with gas chromatography-mass spectrometry. *Journal of Dairy Research* **2002**, *69*, 163-171.
117. Alimentarius, C., Codex standard for milkfat products. In *Codex Stan 280-1973*, Codex Alimentarius: Rome, Italy, 2010; p 3.
118. Ganguli, N. C.; Jain, M. K., Ghee - its chemistry, processing and technology. *Journal of Dairy Science* **1973**, *56*, 19-25.
119. Yadav, J. S.; Srinivasan, R. A., Advances in ghee flavour research. *Indian Journal of Dairy Science* **1992**, *45*.
120. El-Rafey, M. S.; Richardson, G. A.; Henderson, J. L., The effect of the method of manufacture of butteroil on its keeping quality. *Journal of Dairy Science* **1944**, *27*, 807-820.
121. Yadav, J. S.; Srinivasan, R. A., Qualitative and quantitative changes in flavour characteristics of ghee made from ripened cream. *Indian Journal of Dairy Science* **1984**, *37*, 350-356.
122. Kim, M. O.; Baltes, W., On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one in the Maillard reaction. *Journal of Agricultural and Food Chemistry* **1996**, *44*, 282-289.

123. Konecny, M.; Cejpek, K.; Cechovska, L.; Velisek, J., Transformation pathways of reductones in the advanced Maillard reaction. *Czech Journal of Food Sciences* **2009**, 27, S149-S152.
124. Hodge, J. E.; Fisher, B. E.; Mills, F. D., Compounds of browned flavour derived from sugar-amine reactions. *Cereal Science Today* **1972**, 17, 34-36.
125. Shaw, P. E.; Tatum, J. H.; Berry, R. E., 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one, A degradation product of a hexose. *Carbohydrate Research* **1971**, 16, 207-213.
126. Yaylayan, V. A.; Mandeville, S., Stereochemical control of maltol formation in Maillard reaction. *Journal of Agricultural and Food Chemistry* **1994**, 42, 771-775.
127. Voigt, M.; Glomb, M. A., Reactivity of 1-deoxy-D-erythro-hexo-2,3-diulose: A key intermediate in the Maillard chemistry of hexoses. *Journal of Agricultural and Food Chemistry* **2009**, 57, 4765-4770.
128. Toyosaki, T., Sakane, Y., and Kasai, M., Effects of medium-chain triacylglycerols (MCT) on the Maillard reaction. *Journal of Food Processing and Technology* **2013**, 4.
129. Pischetsrieder, M.; Schoetter, C.; Severin, T., Formation of an aminoreductone during the Maillard reaction of lactose with N-alpha-acetyllysine or proteins. *Journal of Agricultural and Food Chemistry* **1998**, 46, 928-931.

Chapter 2 Volatile compound analysis

2.1 Introduction

This chapter focuses on the method development for analysis of volatile compounds in ghee and model systems, using gas chromatography paired with mass spectrometry (GCMS). Optimising an appropriate analytical method was a crucial step for this project; a consistent and automated method, able to analyse large numbers of samples was needed.

The objective for the analysis was monitoring the changes in the volatile compound profiles when the external reaction conditions and the internal structure of the model system were changed. For this reason the method chosen needed to be functional across many different sample types and sample matrices. The samples being analysed contained a large number of different compounds. The compounds being detected exhibit a range of properties that will influence the analysis and there was a need to detect the compounds at both high and low concentrations.

The screening of the samples for changes in volatile compounds under multiple conditions required a high throughput method that could be automated for experimental efficiency.

2.2 Analysis of volatile compounds

There are various methods of analysing volatile compounds; which is most appropriate depends on the nature of the analysis required, the objective of the analysis and the sample being analysed. The method of sample preparation also needs consideration when determining the most appropriate analytical technique.

2.2.1 Analysis techniques

Due to the volatile nature of the flavour compounds being investigated, the use of GCMS has been commonly used (1-7) and widely applied for the analysis of food (7-10) including ghee and related products (2, 11-13) along with model Maillard reaction systems (14). GCMS has been coupled with both direct injection (2) and head space (12, 13) techniques for these types of samples. Mass spectrometry is commonly used as the detector for the analysis of Maillard reaction products. There are three main uses of GCMS to investigate the Maillard reaction (7): identification of unknown

compounds, quantification of known compounds and the study of Maillard reaction mechanisms. The comparison of unknown compound mass spectra to vast libraries of standard spectra is a useful tool for identifying compounds (13). However, it is always best to identify the compound based on the spectrum and retention time of an authentic compound run through the same system.

Although commonly utilised for this type of analysis there were other options besides the GCMS for analysis such as liquid chromatography-mass spectrometry (LCMS) (15), nuclear magnetic resonance (NMR) (15) electrochemistry (16, 17), capillary electrophoresis (CE) (18), fluorescence (19, 20) and ultraviolet spectroscopy (UV) (21). When studying both Maillard and caramelisation reaction products in foods it is necessary to separate them from each other to accurately account for reaction contributions (22). In systems where both Maillard and caramelisation reactions are important and need to be monitored, the disappearance of the reducing sugar (e.g., glucose) and amino acid groups along with increasing UV absorbance, browning levels and protein reticulation have been monitored (23). High performance anion exchange chromatography was used to monitor glucose loss and protein reticulation was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (23). Isotopic labelling with C¹³ and N¹⁵ allows for tracking by GCMS to monitor reaction pathways and fragmentation products (24).

For odour active compounds the technique of gas chromatography-olfactory can be a valuable tool (25). This technique is useful for odour compounds where the molecule may give a large GC peak area as generated by a detector but does not correspond to high odour intensity. The same can be said for a compound with a small peak area coupled with high odour intensity. There is, however, a need to assess the impact of the sample matrix on the odour intensity due to the complex nature of interactions between flavour molecules and other food components. The compounds are detected by the human olfactory system providing sensory analysis as well as analytical (typically a second detector is also used). A detection outlet of the GC is connected to a sniffing port that is located in an isolated environment to eliminate effects of outside odours. The results are limited by the limit of detection associated with an individual evaluator's sense of smell (25). This technique would be very useful for later studies in this field, to correlate the presence of specific volatiles compounds with perceived flavour in ghee.

An interesting technique used for monitoring the Maillard reaction is the use of electrochemical monitoring (16). The Maillard reaction intermediates are a viable electrochemical system, as the reductones formed are known to be electrochemically active. The use of a platinum working electrode and an Ag/AgCl (saturated KCl) reference electrode allows redox potentials to be measured. Based on a model system using β -alanine and carbohydrates, an increasingly negative cell

potential was observed over time as browning increased. This was indicative of the formation of chemically reducing species (16).

For this study GCMS was chosen as the most appropriate analytical technique because of the ability to screen for a large range of volatile compounds using an automated system. GCMS allowed compounds that were present in ghee and any model systems to be detected at a range of different levels throughout the study and the sample analysis to be automated. The GCMS can also be coupled with a range of sample preparation techniques.

2.2.2 Sample preparation techniques

A variety of sample preparation techniques are available for use depending on the analytical technique chosen. Each technique has its advantages and disadvantages in terms of sample handling, interferences, automation and time considerations.

Solid phase micro extraction (SPME) can be paired with headspace GCMS for analysis of volatile compounds. Headspace has been particularly useful for dairy products, e.g., ghee (12, 13) as the nature of the food component matrix is complex and has the potential to affect the release or retention of the volatile flavour compounds (26). Headspace SPME provides a clean sample for desorption onto the column and allows for high throughput sampling with minimal sample preparation (27, 28). It also allows the analytes to be removed from complex matrices. Care needs to be taken using SPME as there are several aspects that need consideration such as the linear range of detection and competition effects on the fibre can lead to biases in quantitative determinations (26). Selection of the optimal fibre can negate some of these effects.

Other sample preparation techniques that could be used include purge and trap, solvent extractions with direct injection or headspace. These techniques can be used along with the introduction of internal standards to the sample to monitor extraction efficiency. The purpose of the analysis is to screen for changes in the volatile compound profile rather than quantitate exact quantities.

There are two options when extracting analytes using SPME: the first is headspace extraction where the fibre is exposed to the volatiles in the gas phase above the sample; the other is liquid phase extraction where the fibre is directly exposed to the analytes in the liquid sample. As the samples in this study were predominantly fat, a liquid based extraction would result in the fibre being covered in fat when introduced into the injection port creating contamination of the column. The best choice was therefore to use a headspace extraction where the volatiles are the only part of the matrix in contact with the SPME fibre.

The size of the sample determines the total quantity of any analyte available to equilibrate into the headspace and then the fibre. A smaller sample has a lower quantity and a larger sample has a higher quantity of analyte. A smaller sample and therefore lower quantity of analyte is less likely to generate competition on the fibre; however, a larger sample can provide a more representative sample of the bulk.

For this analysis a method using SPME for the sample preparation was chosen since it could be automated and introduce analytes directly from the sample to the GCMS column without extra handling steps. Due to the variety of compounds being detected within the samples and the requirement to monitor many of them over the course of the study a quantitative detection method was not feasible. This method was adapted from one previously developed in-house (29).

2.3 Standard samples

For the purposes of this study, a qualitative screening method was required for a variety of Maillard reaction compounds. Internal standards or standard curves were not used for this study as there was a wide range of compounds to be monitored. An internal standard would have been difficult to accurately add to each sample after cooking and generation of standard curves for each monitored compound would be time consuming. However a check on the system functionality was required along with a sample for optimising the system. A commercial ghee sample made by Fonterra, dairy flavour concentrate (DF300) and blank vials were used for the system checks.

2.3.1 Blank vials

Blank vials served the purpose of a control for system contamination. A blank sample should contain only peaks that are attributed to volatile compounds on the fibre as shown in figure 2.1. Any sample carry over would highlight a need to change the fibre, indicating that the extraction/desorption process was not optimal or that the system was overloaded. The blank sample also conditioned the fibre to remove any adsorbed volatile compounds from the lab environment.

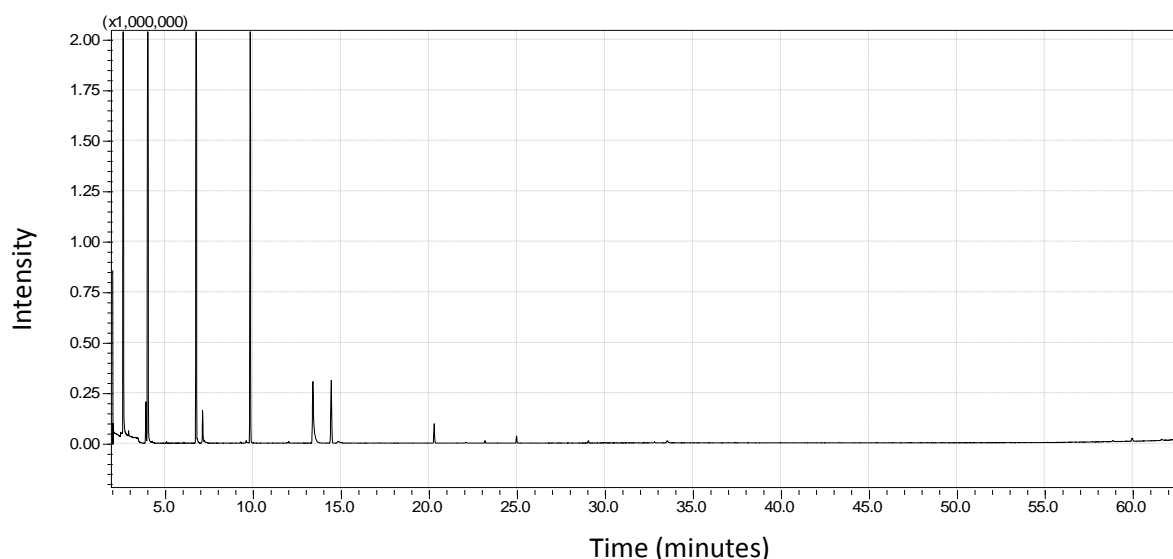


Figure 2.1 A typical chromatogram from a blank vial run using the optimised method (chapter 8 section 2.5).

The intensity of the silane compounds detected in the analysed samples fluctuated greatly, this fluctuation could be due to the preferential desorption of analytes from the fibre over desorption of the silanes. This fluctuation can be seen when the peaks at 7 and 10 minutes in figure 2.1 are compared with those detected at the same times in figure 2.2. It is also possible that any silanes likely to desorb from the fibre are desorbed when the blank sample is run and therefore a lower quantity of silanes are present to desorb when subsequent samples are analysed used the fibre.

2.3.2 Dairy flavour concentrate

Dairy flavour concentrate (DF300) is a commercial ghee product (chapter 8, section 8.2.2) and was used as a reference material at the start and end of each run to check that the system was working correctly. Samples were also run in the middle of large batches as a system check during the batch analysis. A typical chromatogram of DF300 is shown in figure 2.2 which shows that the sample contains a range of Maillard reaction compounds, methyl ketones, acids and silanes from the fibre.

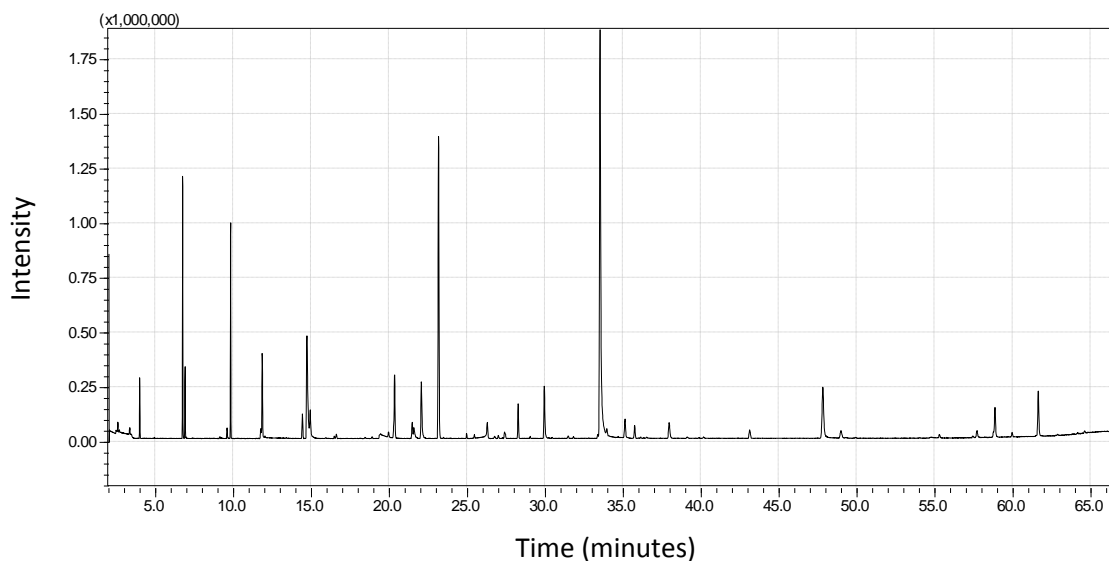


Figure 2.2 A typical DF300 chromatogram run using the optimised method (chapter 8 section 2.5).

2.4 Gas chromatography optimisation

2.4.1 Column selection

The compounds that are commonly associated with ghee and related dairy products such as maltol, furfural, and HMF have varying degrees of acidity, are polar and are volatile with a range of boiling points. This suggested that a polar column would be suitable for the analysis of these volatile organic compounds. Most publications dealing with the analysis of the Maillard compounds, both in ghee and model systems, use a 'wax' type stationary phase in the column (2, 13).

The column used on the instrument was an EC-Wax column made by Grace (Part no. 19655). The EC-Wax column is commonly used for analysis of fatty acid methyl esters (FAMES), polar solvents, alcohols, flavours, fragrances, glycols and aromatic compounds (30). The stationary phase of the column is 100% polyethylene glycol, the structure is shown in figure 2.3 (30).

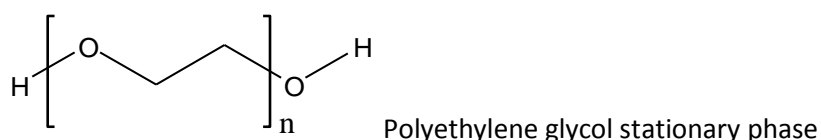


Figure 2.3 The stationary phase of the EC-Wax column: Polyethylene glycol (31).

The dimensions of the column relate to the phase thickness, internal diameter of the column and the length of the column. The chosen column was a 30 metre long column that had an internal diameter

of 0.25 mm with a film thickness of 0.25 μm . Each of these parameters has the ability to change the efficiency, capacity, resolution, and retention of analytes on the column. The dimensions of the column used were fairly typical dimensions that provide good resolution although the column had a smaller sample capacity than thicker film columns (32). The narrow diameter of such columns coupled with the thin and evenly distributed stationary phase provides fast mass transfer of analytes between the stationary and mobile phases. The low flow rate was also suitable for the coupling with mass spectrometers (32).

2.4.2 Temperature gradient

An isothermal method would not be suitable for analysis of ghee as there are a large number of analytes to be separated and a lengthy analysis time is undesirable as the peaks will broaden. A temperature gradient allows a greater number compounds to be separated in a shorter amount of time. The initial starting temperature for the column was 50 °C. The minimum column temperature is 40 °C (32); below this temperature the stationary phase (polyethylene glycol) will be frozen. A starting temperature of 50 °C ensures the stationary phase is liquid. The final temperature must remain below the maximum temperature limit for the column of 280 °C. However, the temperature also needs to be high enough to elute all of the compounds from the column. Preliminary sample runs demonstrated that with a final temperature of 220 °C there was no carryover of slow eluting compounds into the next sample run.

Using the start (50 °C) and end (220 °C) temperatures, a series of constant slope gradients were run. The runs ranged from temperature increases of 1 °Cmin⁻¹ up to 6 °Cmin⁻¹. There was a 5 min hold time at 50 °C and again at 220 °C; these holds were applied to allow the compounds time to separate out before being forced along the column by increasing temperatures, and finally to ensure all compounds had eluted from the column before the start of the next run. Experimental details can be found in chapter 8, section 8.2.6.

At 1 °Cmin⁻¹ (figure 2.4) the time taken to run the sample was considered too long for use as a high throughput method and the quality of chromatogram was low, there were also large tailing peaks coming out at the end of the run. The temperature gradient was increased incrementally by 1 °Cmin⁻¹ up to a gradient of 6 °Cmin⁻¹ (figure 2.5). At a gradient of 6 °Cmin⁻¹ the analytes eluted from the column six times faster and as such there was a lack of separation across the entire chromatogram. Although compounds can be separated using mass spectrometry techniques, it was preferable to separate the compounds using chromatography.

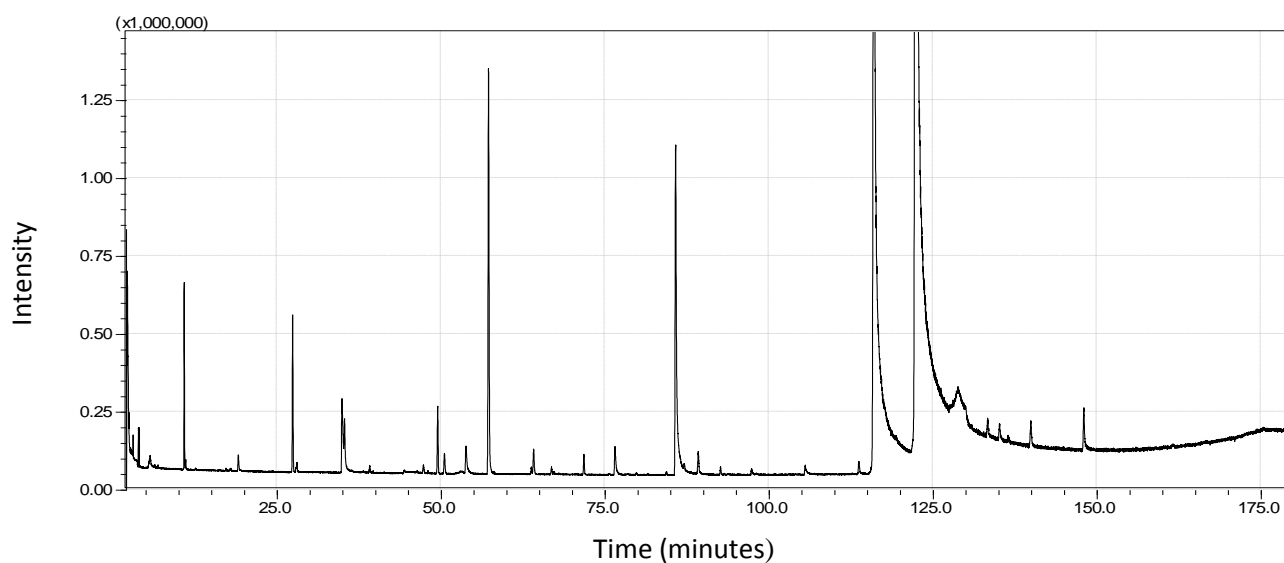


Figure 2.4 Temperature gradient set at $1\text{ }^{\circ}\text{Cmin}^{-1}$.

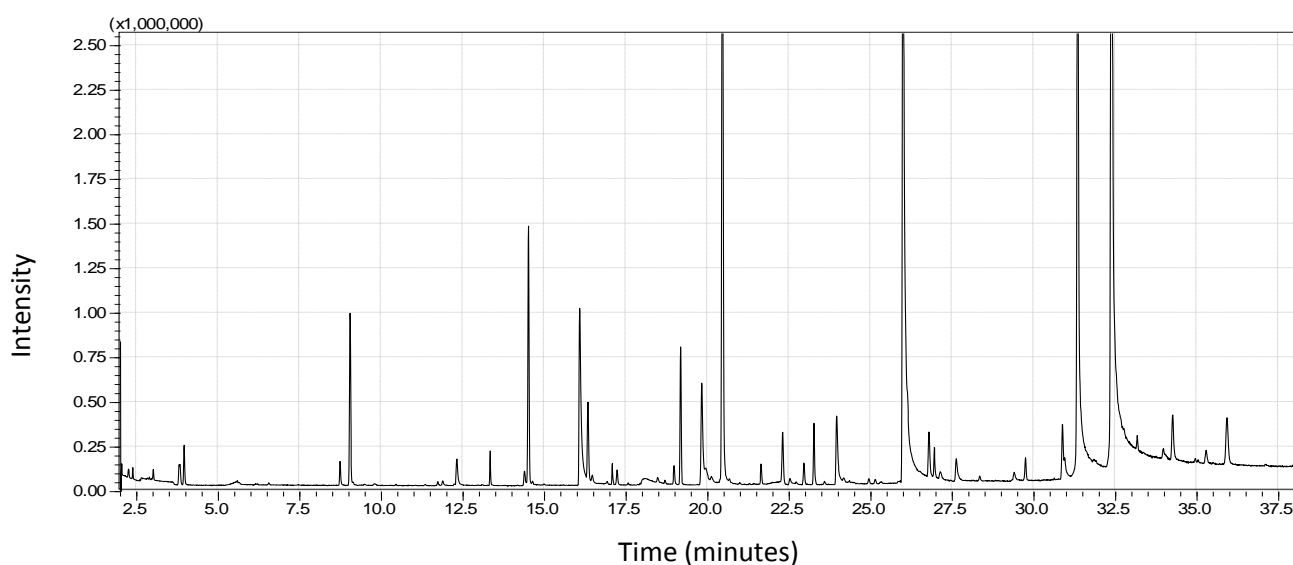


Figure 2.5 Temperature gradient set at $6\text{ }^{\circ}\text{Cmin}^{-1}$.

It was found that the optimum gradient was between $3\text{ }^{\circ}\text{Cmin}^{-1}$ and $4\text{ }^{\circ}\text{Cmin}^{-1}$ after considering the time for separation and the peak resolution achieved. By incrementally increasing the gradient at $0.25\text{ }^{\circ}\text{Cmin}^{-1}$ from $3\text{ }^{\circ}\text{Cmin}^{-1}$ to $4\text{ }^{\circ}\text{Cmin}^{-1}$ the chromatogram in figure 2.6 was achieved when the gradient was $3.25\text{ }^{\circ}\text{Cmin}^{-1}$.

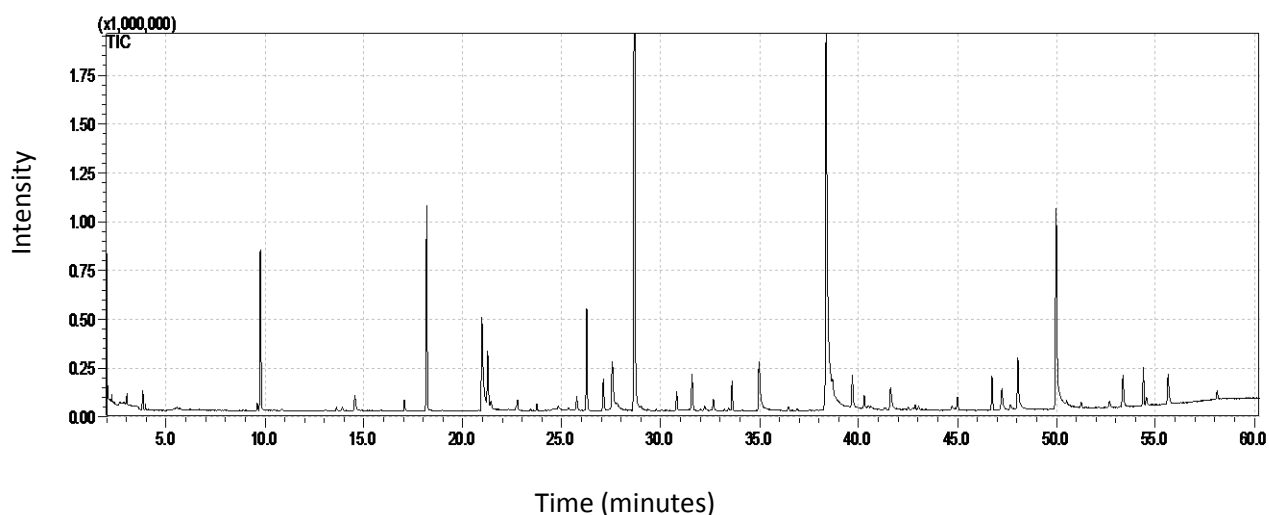


Figure 26 3.25 °Cmin⁻¹ temperature gradient.

Using the results from the incremental gradient experiments, the elution of compounds over the course of analysis was able to be adjusted to reach optimal separation (figure 2.7).

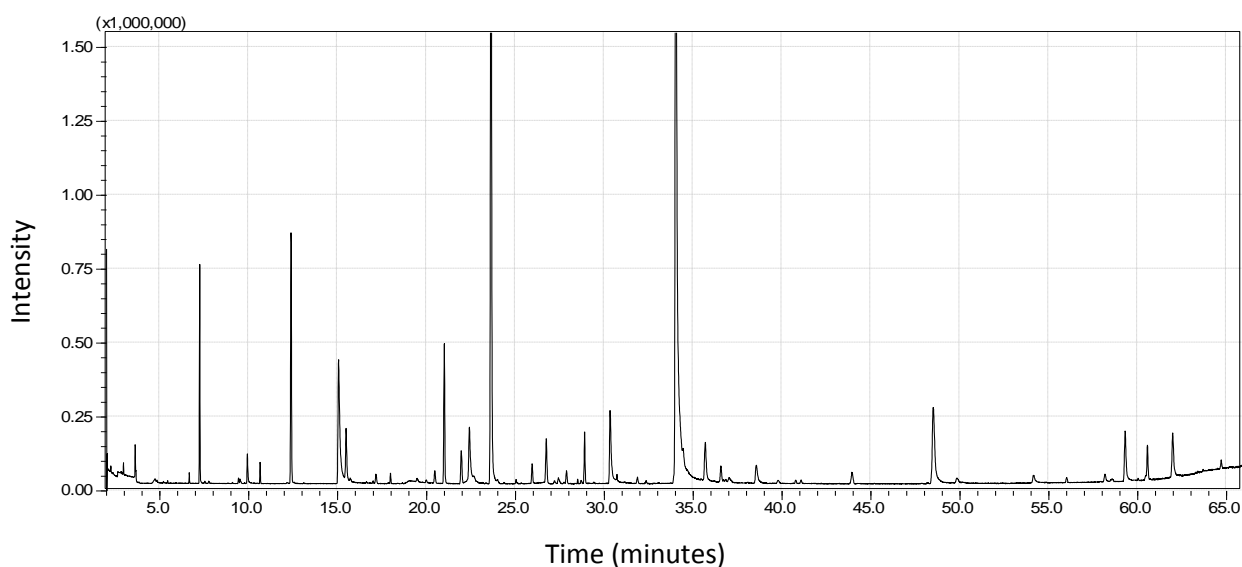


Figure 2.7 Chromatogram achieved using the final temperature gradient (table 2.1).

The final gradient (table 2.1) achieved a separation of virtually all compounds to a baseline level with a couple of exceptions that could be separated using mass spectrometry.

Table 2.1 Final temperature profile of GC column

Initial temperature (°C)	Final Temperature (°C)	Rate of change (°Cmin ⁻¹)	Hold time (min)
50	50	0	2
50	95	6	0
95	95	0	5
95	150	3.25	0
150	170	1	0
170	220	4	0
220	220	0	2

2.4.3 Instrument parameters

The compounds are desorbed from the SPME fibre in the injection port and swept onto the column by the carrier gas (helium in this case). The temperature in the injection port needs to be hot enough to volatilise all of the compounds as any that are not volatilised at this point will collect on the front of the column and in the glass liner as impurities. The injection port temperature is also the temperature at which the compounds are desorbed from the SPME fibre. The injection temperature of the method was selected as 220 °C. The injection temperature is required to be high enough that the analytes are desorbed from the SPME fibre and remain volatile as they are swept onto the column (33, 34). The injection mode can either be split so that only a portion of the volatiles are swept onto the column or splitless where all of the volatiles are swept off the fibre and directly onto the column (35). A split ratio was chosen depending on the amount of analytes present on the fibre and the response factors of these compounds to avoid column overloading whilst still having enough of the analytes to achieve a representative chromatogram.

The initially selected parameters were an injection temperature of 220 °C and a split injection with a ratio of 1:10. Three split valve settings were investigated: splitless, split ratio of 1:10 and split ratio of 1:100. Figures 2.8 – 2.10 show the results of the same DF300 sample run at these three different split conditions. After running the variations the decision was to leave the split ratio at 1:10 and the injection temperature at 220 °C.

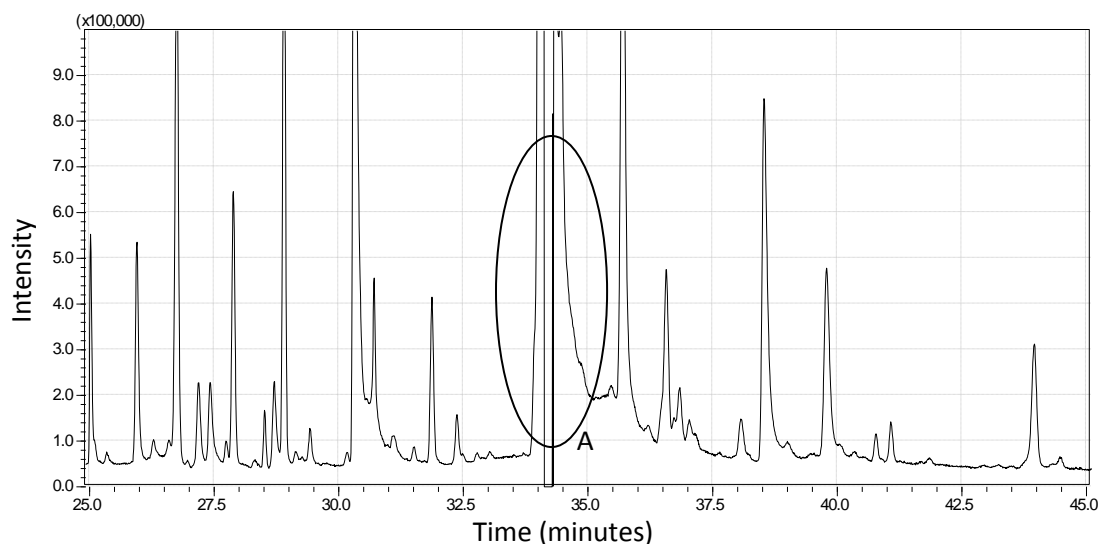


Figure 2.8 The oval marked A denotes overloading of the column as a result of a splitless injection.

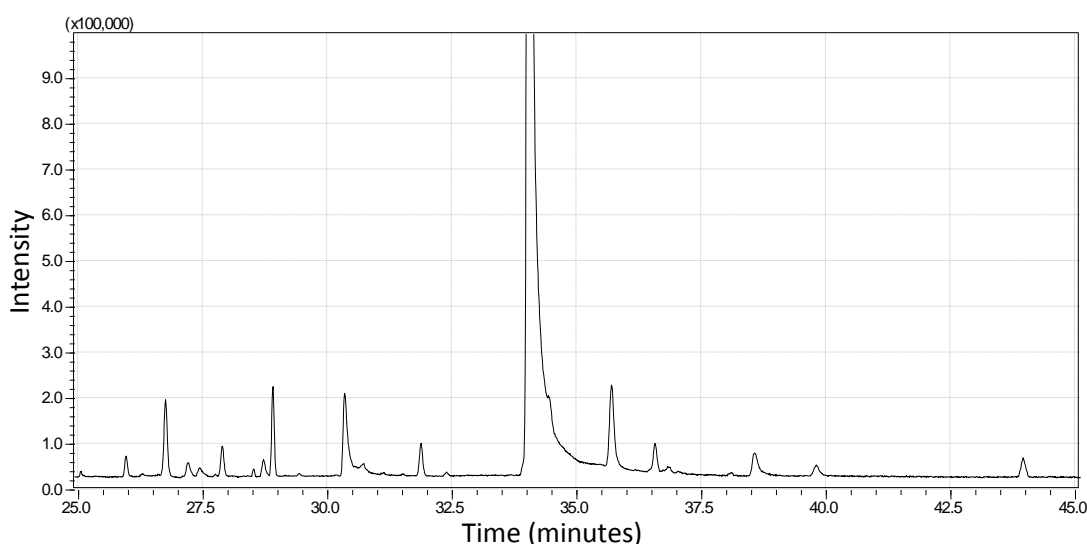


Figure 2.9 Split injection with a 1:10 ratio.

Using the splitless injection ratio 100% of the analytes desorbed from the SPME fibre were introduced on to the column for separation. The large quantity of analytes eluting at ~33-36 minutes overloaded the column and generated a response that was outside of the mass spectrometer detection limits. This was evident by the sudden decrease and increase in peak intensity accompanied by a gap in the peak where the intensity was zero, this is highlighted with an oval marked A in figure 2.8. The split injection with a ratio of 1:10 (figure 2.9) provided an improvement in chromatogram quality relative to the splitless injection (figure 2.8). The peaks were sharper along with a smoother baseline, less peak tailing and the detector was not overloaded. However, a further

reduction in the split ratio to 1:100 (figure 2.10) meant that not enough of the analyte was loaded onto the column leading to small peaks that were sometimes lost in the baseline noise.

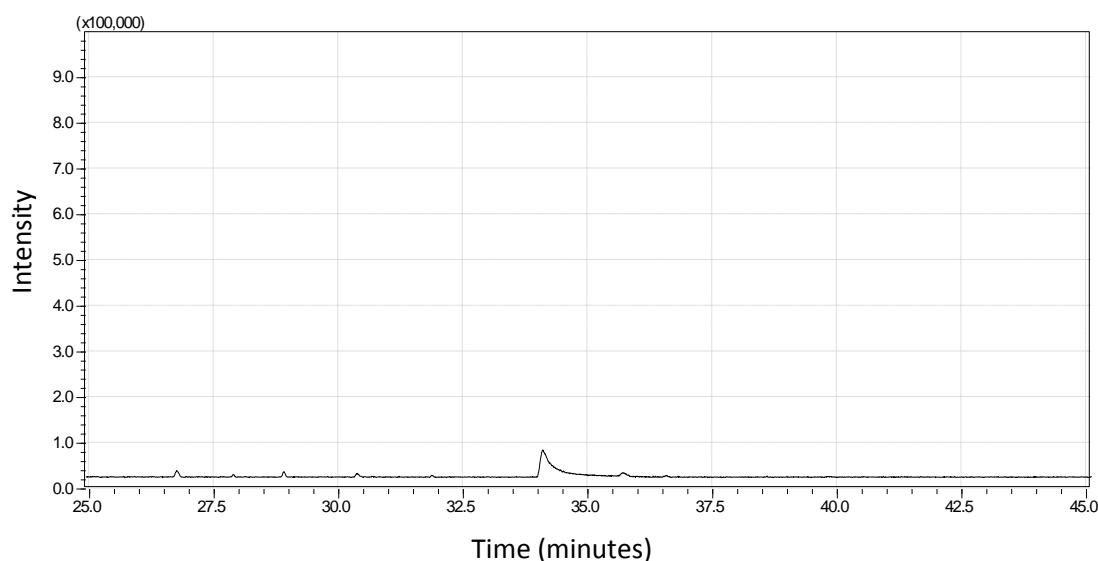


Figure 2.10 Split injection with a 1:100 ratio.

Although there are various options for a carrier gas, the GCMS instrument was already set up with helium as the carrier gas and it was not thought that there was any analytical advantage to be gained by changing this.

2.5 Solid phase microextraction (SPME) optimisation

After selection of a suitable SPME fibre there are two steps that are carried out during SPME: the solute adsorption from the sample matrix onto the fibre and the transfer of these analytes (desorption) in the injection port both of steps were considered during the optimisation.

2.5.1 SPME fibre selection

The role of the fibre is to collect the volatile compounds from the sample, and then retain those compounds until they are desorbed in the injection port. The fibre must be selected for the compounds to be extracted from the headspace. Fibres have a range of coatings each with different polarities ranging from non-polar to polar. For some compounds, such as many Maillard reaction compounds, that may have mixed polarity and mixed volatility a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre is suitable and can be used for the extraction of compounds with a molecular mass range of 50-300 Da. The thickness of the fibre coating

influences the adsorption/desorption properties of the fibre. Thin coatings (10 μm) offer retention of semi-volatile compounds with fast diffusion capabilities while a thicker coating (100 μm) will permit the retention of highly volatile compounds. The chosen fibre (PDMS/DVB coating) had a thickness of 65 μm . This fibre was supplied by Sigma-Aldrich and is considered suitable for volatile compounds that contain amines, alcohols and other volatile polar groups (36).

2.5.2 Extraction parameters

During the headspace sampling the analytes are distributed between three phases: the fibre coating, the headspace and the sample (liquid or solid) phase (36). Each compound equilibrates between these three phases based on the kinetics of mass transport and the properties of the compound. In the standard sample (DF300) there are many compounds each of which will have a different equilibrium between the phases. The distribution between the phases is temperature dependent and is also influenced by pH, ionic strength and the presence of organic solvents. At a higher temperature, analytes will move favourably from the sample into the headspace. However, this needs to be balanced with the change in the equilibrium between the headspace and the fibre which also favours the headspace at higher temperatures. The extraction time is related to the extraction temperature as the equilibria are established at different rates based on the temperature of the system.

Different extraction temperatures were tested using a fixed extraction time of 20 minutes and the results demonstrated the effect that the properties of the individual components have on finding an optimal method. An extraction temperature of 60 °C was used as a starting point (29, 37, 38) with temperatures of 50 °C (38, 39) and 70 °C (38) also being tested. As the temperature was increased from 50 °C to 60 °C and then to 70 °C there were distinct differences between the peak areas. The peak areas of acetic acid and furfural decreased as the temperature was increased from 50 °C (figure 2.11a) whereas some compounds (figure 2.11b) including undecanone appeared to have a linear increase in peak height with temperature, other compounds (figure 2.11c and d) had a larger peak area when extracted at 60 °C.

It was decided to keep the extraction temperature at 60 °C, as extraction at this temperature provided adequate amounts of all compounds for analysis, and had previously been used for the analysis of ghee (29), and for the extraction of compounds from emulsions (37). The temperature is also high enough to ensure that any fats that are found in solid state at room temperature will be in a liquid state.

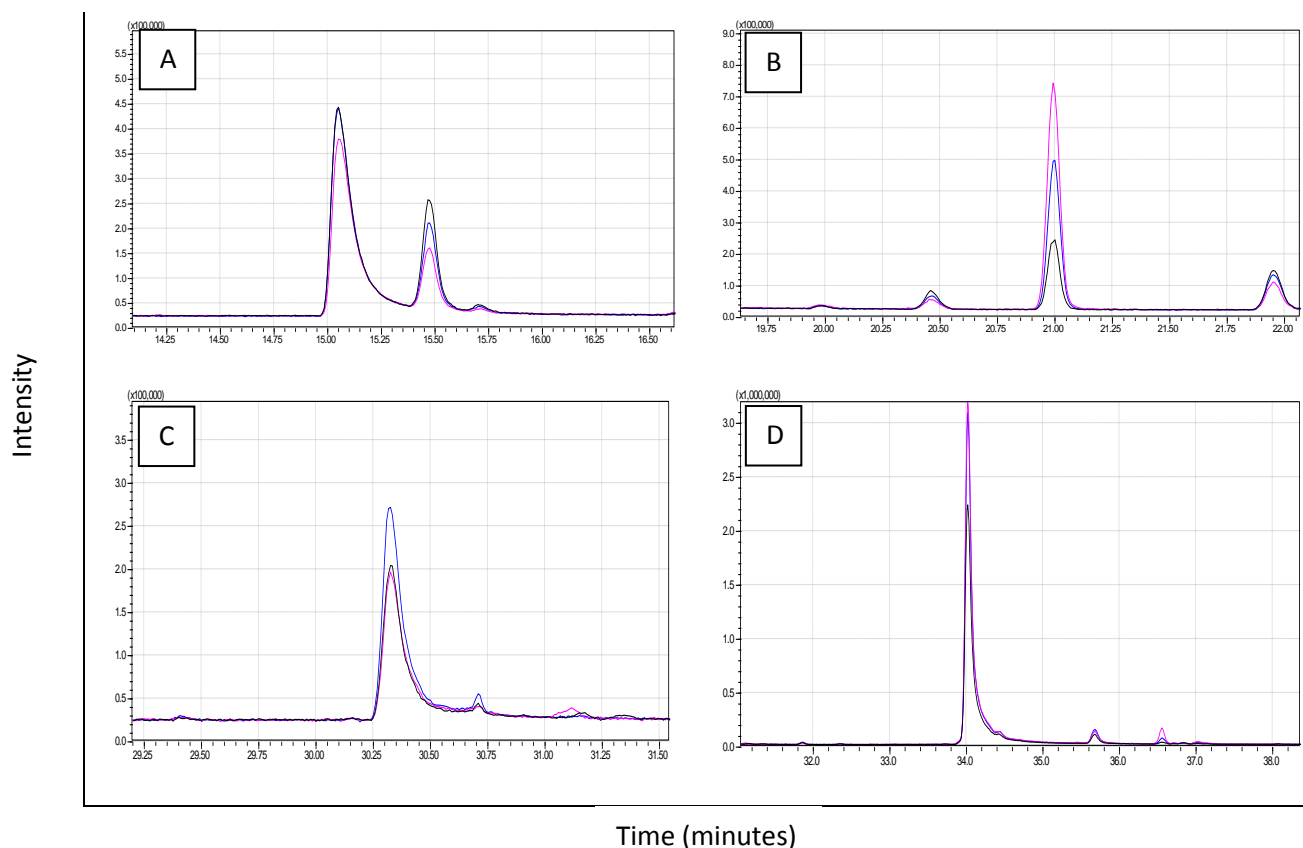


Figure 2.11 Influence of extraction temperature on selected DF300 compounds: A, acetic acid (left) and furfural (right); B, undecanone (21 minutes); C, hexanoic acid; D, maltol. Black, 50 °C; blue, 60 °C; pink = 70 °C.

The extraction time used to investigate the influence of changing extraction temperature was 20 minutes. The extraction time was varied to include three time points (chapter 8, section 8.2.6.4) of 10, 20 and 30 minutes (29, 38) using the optimised temperature of 60 °C. Reducing the extraction time to 10 minutes decreased the amount of many compounds that were extracted with the exception of acetic acid and furfural (figure 2.12). On the other hand, increasing the extraction time to 30 minutes increased the extracted amount of only a few compounds such as undecanone (figure 2.12). The decision was made to utilise the 20 minute extraction time.

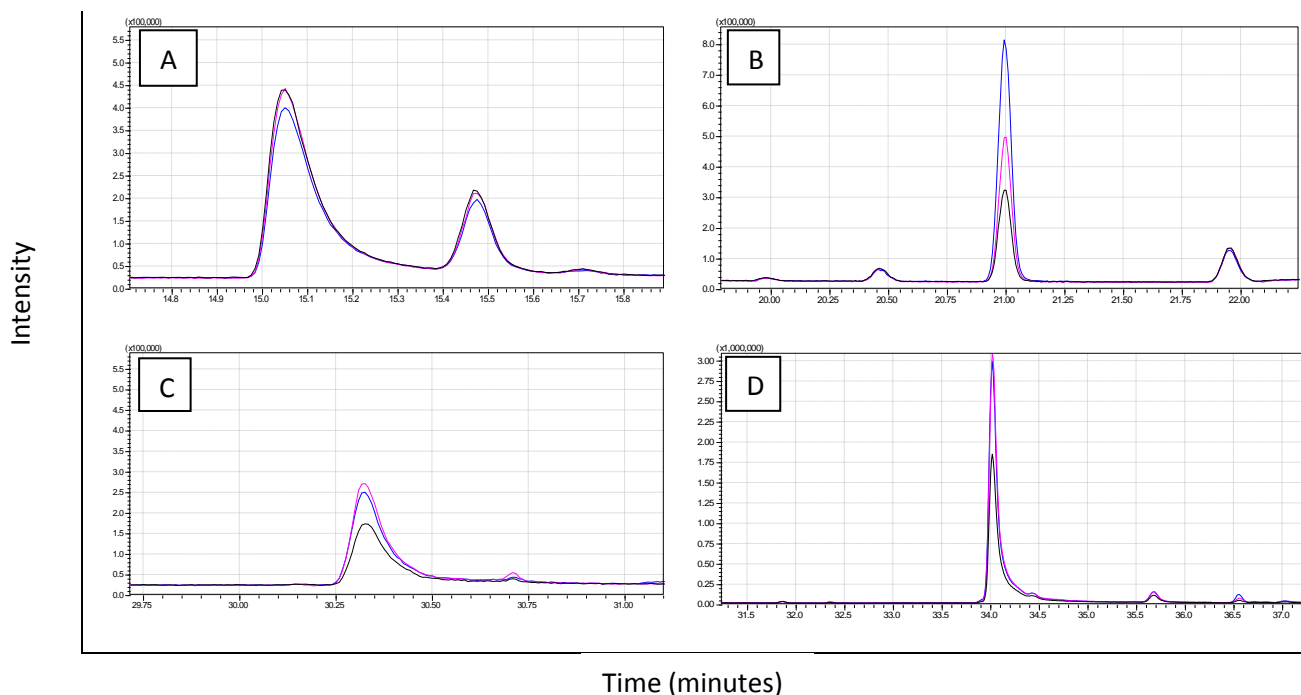


Figure 2.12 Influence of extraction time on selected DF300 compounds: A, acetic acid (left) and furfural (right); B, undecanone (21 minutes); C, hexanoic acid; D, maltol. Black, 10 minutes; pink, 20 minutes; blue, 30 minutes.

Given sufficient time to test a wider range of time/temperature combinations it may have been possible to achieve a more optimal system. However, the result achieved using the 20 minute extraction at 60 °C was deemed suitable for this work which centred on the analysis of a wide range of compounds at a range of different concentrations.

2.5 Mass spectrometry

Mass spectrometry requires a gaseous sample of high temperature to be introduced into the ionisation chamber. The ionisation technique used in this instrument is electron ionisation (EI). High voltage electrons are accelerated as a beam (70 eV) into the gaseous analyte molecules; this can impart enough energy to generate a charged molecule, or ion, by the removal of an electron. The energy and instability of the molecular ion can lead to fragmentation in a repeatable pattern. Molecular ions will fragment in the same pattern under the same conditions. These charged fragments then pass through the mass analyser. The sampling rates (Hz) were 0.5 (scan) and 0.2 (single ion monitoring, SIM) with the mass range being sampled between 40 and 350 Daltons. All of the volatile compounds likely to be found in ghee and any model samples are small molecules with a

molecular mass below 350 Da. The interface between the GC column and the mass spectrometer was set at 230 °C and the ion source temperature at 200 °C. These temperatures ensure that there was no condensation of volatile compounds inside the mass spectrometer. The MS conditions used were taken from a method previously developed at Fonterra (29).

2.6 Data analysis

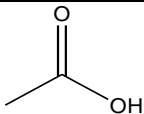
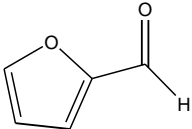
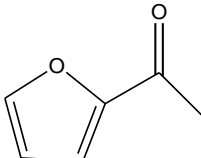
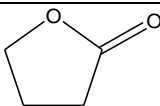
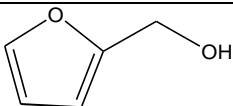
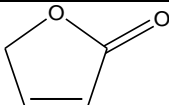
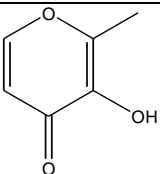
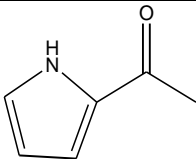
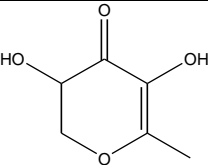
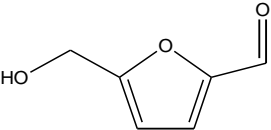
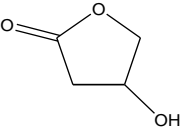
This project was concerned with changes that occurred in flavour formation when the matrix of the sample being cooked was changed; as such there was no requirement to know the absolute quantity of a compound present. The absolute quantities of any particular compound are not as important as the relative changes in formation of compounds in one matrix as compared with another e.g., an aqueous matrix versus an emulsion matrix. Studies that have used quantitative methods for ghee analysis were primarily concerned with the quantity of each measured compound in certain types of ghee (2, 13). Of course, the use of external or internal standards for any compounds that are of particular interest could allow most methods to become quantifiable with either internal or external standards.

2.7 Compound identification

The Wiley compound library (Wiley 7) was used for tentative identification of peaks in the mass spectrum of each sample. These identifications were based on comparisons made to reference spectra in the library. Throughout this work the peaks of interest were identified using this identification system. A range of compounds were identified in the DF300 chromatogram including Maillard reaction products, methyl ketones, acids and silane compounds derived from the fibre (figure 2.13).

The range of compounds present in each sample includes Maillard (and caramelisation) reaction compounds plus compounds from the fats. Maillard reaction compounds were the compounds that were of most interest for this thesis. Based on the peak picking (figure 2.13) a group of eleven compounds were selected for tracking throughout the rest of the experiments (table 2.2). Monitoring of all the compounds was still carried out but these eleven compounds were the main focus since they have a range of flavour attributes (40) (table 2.2) and are formed by a variety of mechanistic pathways.

Table 2.2 Compounds identified in DF300 samples and chosen for monitoring

Name	Structure	Flavour
Acetic acid CAS: 64-19-7		vinegar, acidic
Furfural CAS: 98-01-1		caramel, almond, sweet
2-Acetylfuran CAS: 1192-62-7		sweet, nutty
Butyrolactone Dihydro-2(3H)-furanone CAS: 96-48-0		milky, creamy, slightly fruity
Furfuryl alcohol CAS: 98-00-1		burnt, sweet, caramel, bitter
2(5H)-Furanone CAS: 497-23-4		buttery
Maltol CAS: 118-71-8		malt, cocoa, caramel, sweet, fruity
2-Acetylpyrrole CAS: 1072-83-9		sweet, tea, nutty
Hyxymaltol 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyranone CAS: 28564-83-2		caramel, burnt sugar
Hydroxymethylfurfural HMF CAS: 67-47-0		buttery, caramel, bitter
Dihydro-4-hydroxy-2(3H)-furanone DHHF CAS: 5469-16-9		None found

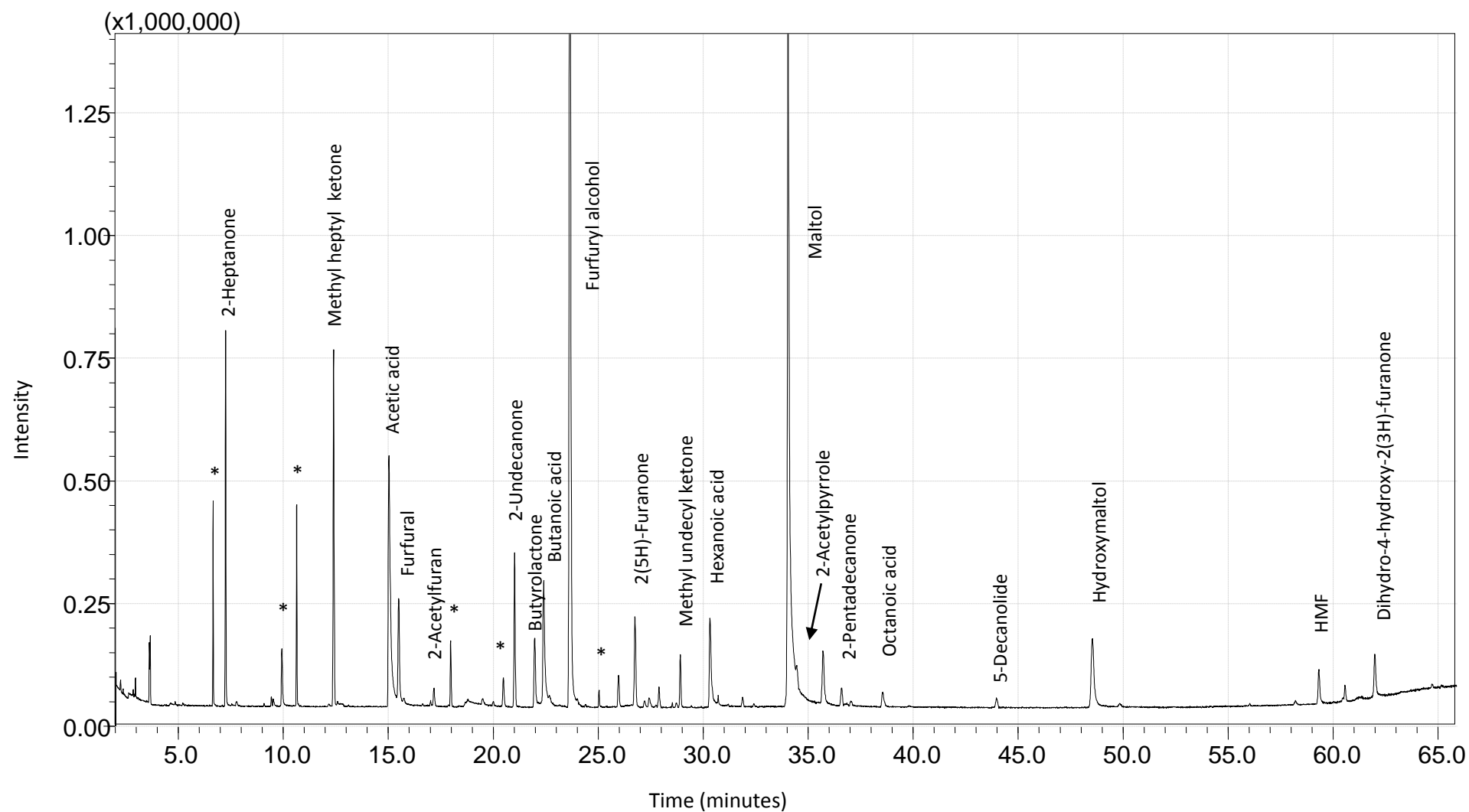


Figure 2.13 Labelled chromatogram of DF300 (not all peaks are labelled) *indicates a peak in the blank due to the SPME fibre.

2.8 Reproducibility and repeatability

The reproducibility of analytical methods using SPME extraction techniques are not as high as when other techniques are used to introduce the analytes to the GC column. The repeatability was tested by triplicate analysis of the same DF300 vial (figure 2.14) and the reproducibility of multiple samples was tested across different days (figure 2.15).

Analysis of the same DF300 vial multiple times (figure 2.14) had a residual standard deviation of <20% across all 11 compounds whilst analysis of multiple DF300 samples across different days had a higher residual standard deviation of up to 50%; although most compounds fell below 30%. Attempts were made to minimise the residual standard deviation by increasing the sample size, adding buffer solutions to alter the pH, altering the SPME extraction and desorption conditions. This demonstrates the need to run control samples for each experiment and randomise sample analysis for large sample sets. Samples run on different days will be more difficult to compare due to the lower degree of reproducibility; however, a set of samples run on the same day can be compared with a greater degree of confidence in the differences.

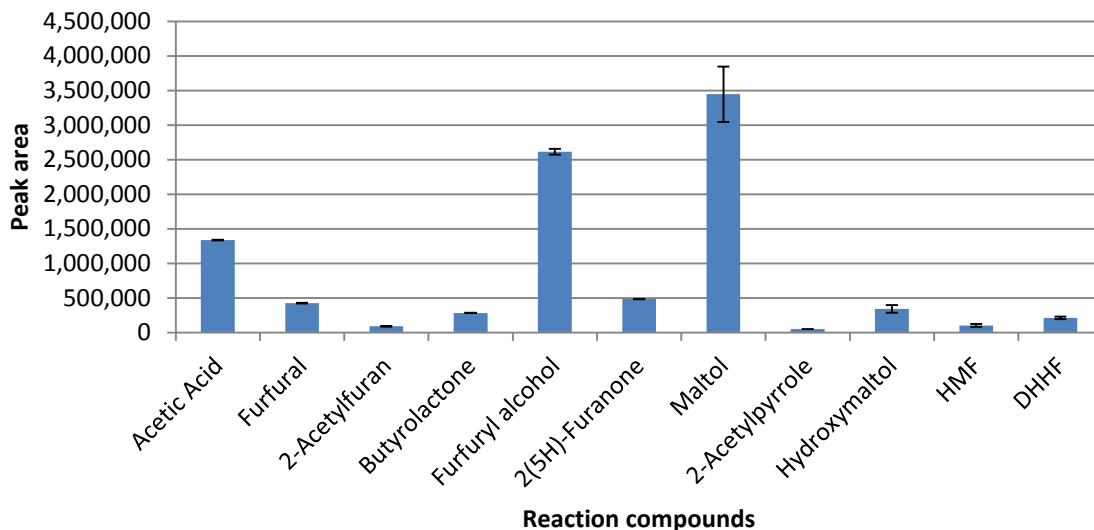


Figure 2.14 Triplicate analysis of a DF300 sample. Error bars represent the standard deviation of the triplicate measurements.

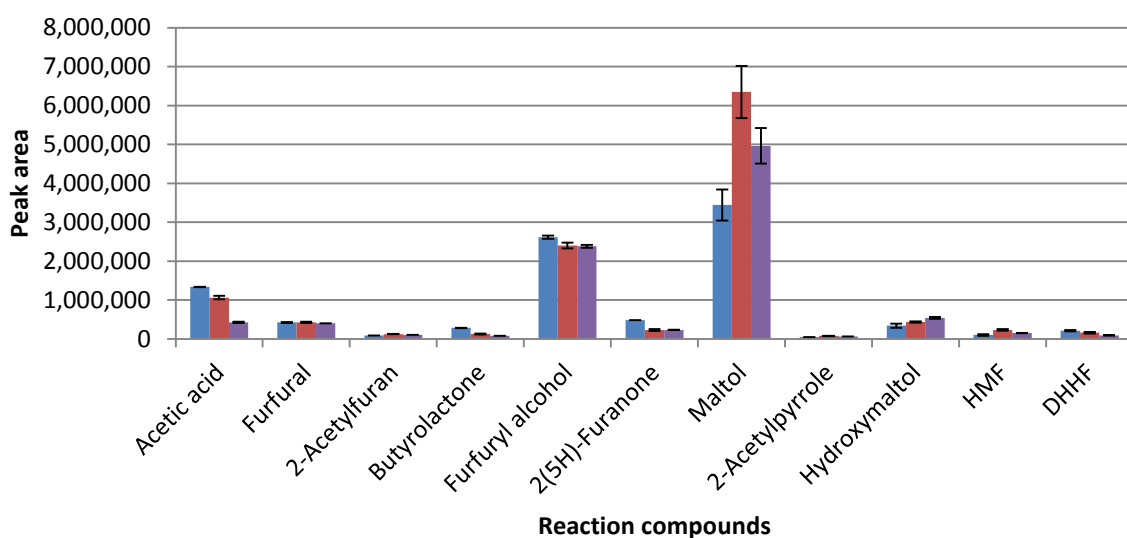


Figure 2.15 Averages of triplicate DF300 samples measured on different days using different DF300 samples each day. Error bars represent the standard deviation of the replicate measurements.

The repeatability of compound retention times was also measured and found that retention times were highly reproducible with a small standard deviation (table 2.3).

Table 2.3 Repeatability of retention times for a DF300 sample analysed in triplicate.

Sample	Acetic				Furfuryl						
	Acid	Furfural	2-Acetylfuran	Butyrolactone	alcohol	2(5H)-Furanone	Maltol	2-Acetylpyrrole	Hydroxymaltol	HMF	DHMF
1	15.03	15.50	17.18	21.98	23.65	26.75	34.04	34.46	48.54	59.32	61.99
2	15.03	15.50	17.18	21.98	23.65	26.75	34.04	34.46	48.54	59.32	61.99
3	15.03	15.50	17.18	21.98	23.65	26.75	34.04	34.46	48.54	59.32	61.99
Average	15.03	15.50	17.18	21.98	23.65	26.75	34.04	34.46	48.54	59.32	61.99
Standard deviation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
%RSD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

2.9 Error Calculations

Error bars used on all graphs are based on the standard deviation of replicate samples.

2.11 Matrix interference

There are various matrix factors, such as pH and the amount of fat present that can affect the final result when analysing for volatile compounds. The headspace GC analysis relies on partitioning of volatile compounds into the gas phase so that when the SPME fibre is exposed the volatiles adsorb onto the surface in an equilibrium between the gas phase and the fibre. However, some matrix components change the partition coefficients, which results in a change in the equilibrium between the sample phase and the gas phase (41).

Liquid extractions can also be used to isolate the compound of interest. However, this method is still prone to matrix effects. Liquid extraction is done by extracting the compounds of interest into another solvent – often a non-polar solvent like diethyl ether, which can then be directly injected onto the column. Caution needs to be taken as this method can cause contamination of the column and injection port if the extraction is not done cleanly. Care needs to be taken particularly with food system samples as they can contain proteins and other non-volatile compounds which can contaminate the column and lead to artefacts.

If two samples have different matrices e.g. if one contains fat and the other does not, but the samples contain the same quantity of a specific analyte there will still be a difference in the partition coefficients of the two samples which will result in differences in final analytical results. Therefore all of the samples in this thesis were matrix matched to ensure analytes were being extracted for analysis from the same matrix regardless of the initial sample matrix by the addition of fat to the aqueous samples after cooking. The samples had either canola oil or AMF added depending on the experiment being carried out. There was no difference in results when using different fats for the analysis of the caramelisation (figure 2.16) or the Maillard reactions (figure 2.17).

The protonation state of a compound can affect its partition coefficient. At different sample pH a compound can have a different protonation state meaning that a salt form of the compound may not be as volatile as its acid or neutral form or may not be volatile at all. The molecules will form an equilibrium between the acid, neutral and salt forms, the favourable direction of the equilibrium depends on the pH of the solution. Cooking cream and the reaction model systems resulted in pH values of 4-5 and this pH was consistently achieved by the final samples. Samples that are analysed at different stages of the cooking time from 2 minutes up to the 20 minute final cook time had pH values below pH 7. The pH of these samples was not adjusted prior to analysis.

The ability to compare the aqueous model samples with the cream samples was crucial to this work and therefore this matrix effect needed to be overcome. Other influences on the matrix could be

components that were added to the system such as salts (including buffers) and emulsifiers added to generate emulsions. The addition of these components to samples of DF300 indicated that there were no significant differences when compared with original DF300 samples. The additional components were tested for interference at two levels: at the level of addition and a level 2-5x higher, depending on the additive.

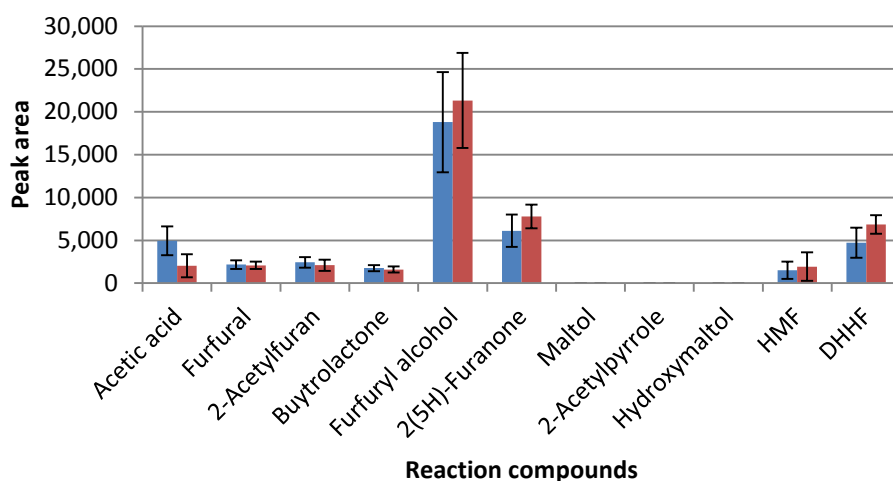


Figure 2.16 The influence of different fats on the analysis of aqueous caramelisation reaction samples. The error bars represent the standard deviation of replicate samples. The samples that were matrix matched with AMF are shown in blue while those matched with canola oil are shown in red.

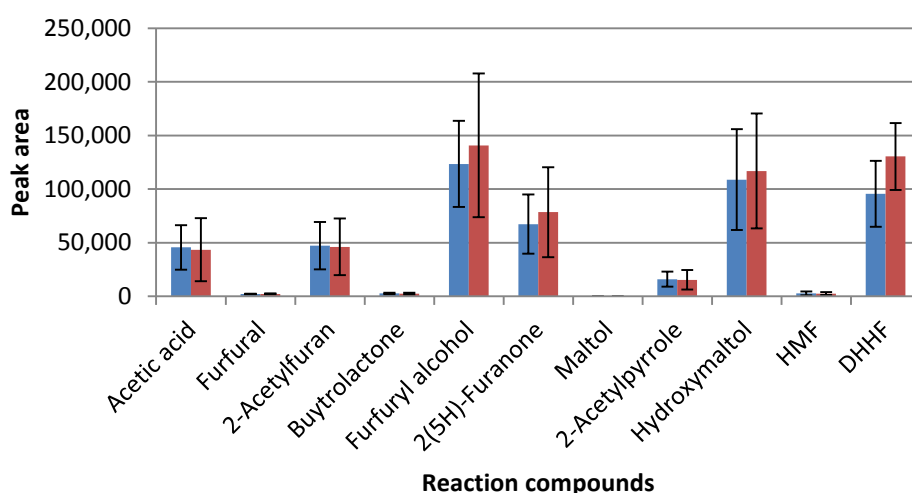
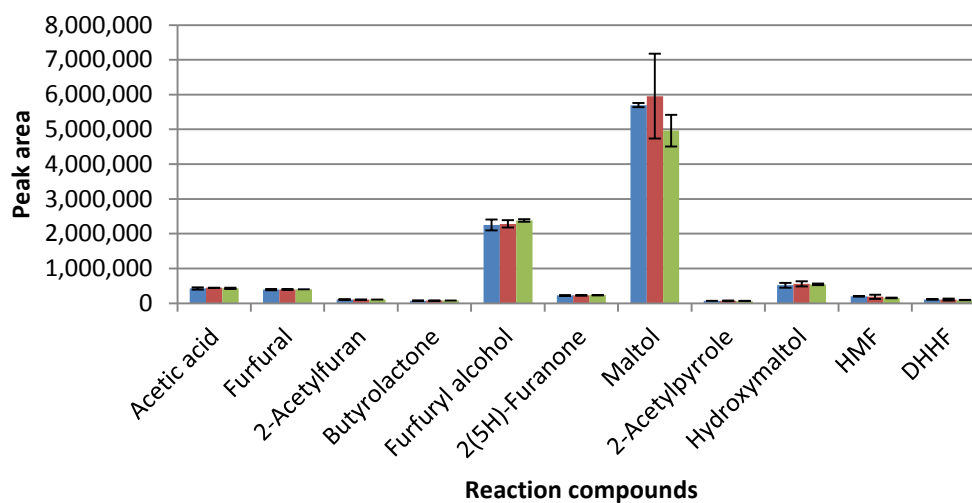


Figure 2.17 The influence of different fats on the analysis of aqueous Maillard reaction samples. The error bars represent the standard deviation of replicate samples. The samples that were matrix matched with AMF are shown in blue while those matched with canola oil are shown in red.



2.18 The influence of PGPR on the analysis of DF300. The DF300 samples containing 3% (blue), 1.5% (red) or 0% (green) PGPR with error bars representing the standard deviation of replicate samples.

The addition of PGPR to DF300 at both 1.5% (experimental level) and at 3% (excess PGPR) did not cause any significant changes to the analysis of the volatile compounds. PGPR is an emulsifier that will be used for the generation of w/o emulsion structure. There is a chance that the PGPR could react with the model system components or that the presence of the PGPR could alter the partitioning of the volatile compounds between the samples, the headspace and the fibre. These results (figure 2.18) suggest that any differences seen in the formation of volatile compounds when PGPR is added will be due to reactions occurring rather than interference of the PGPR with the partitioning of the volatile compounds. The same scenario could happen with the addition of the o/w emulsifier Tween 60 (figure 2.19); the results indicate that there will be no interference with the analysis of the volatile compounds. This does not rule out the possibility that the Tween 60 can participate in the reactions.

It is expected that the addition of sodium chloride (NaCl) and sodium phosphate buffers could influence the Maillard and caramelisation reactions when added to a model reaction system. The addition of these salts could also interfere with the analysis by altering the partitioning of the volatile compounds between the sample, headspace and fibre. The addition of NaCl (figure 2.20) leads to some small changes in the levels of detected volatile compounds; however, the changes are smaller than the calculated error and therefore unlikely to be significant. The sodium phosphate buffer (figure 2.21) generated similar small changes in the results when DF300 containing the buffer

was analysed and it was concluded that the presence of the buffer was not causing any interference with the analytical method. Any differences in the formation of volatile compounds seen when comparing results of samples cooked with and without NaCl or sodium phosphate buffer can be assumed to be generated through the participation of NaCl or sodium phosphate buffer in the Maillard and caramelisation reactions as there is no significant interference of these components in the analysis.

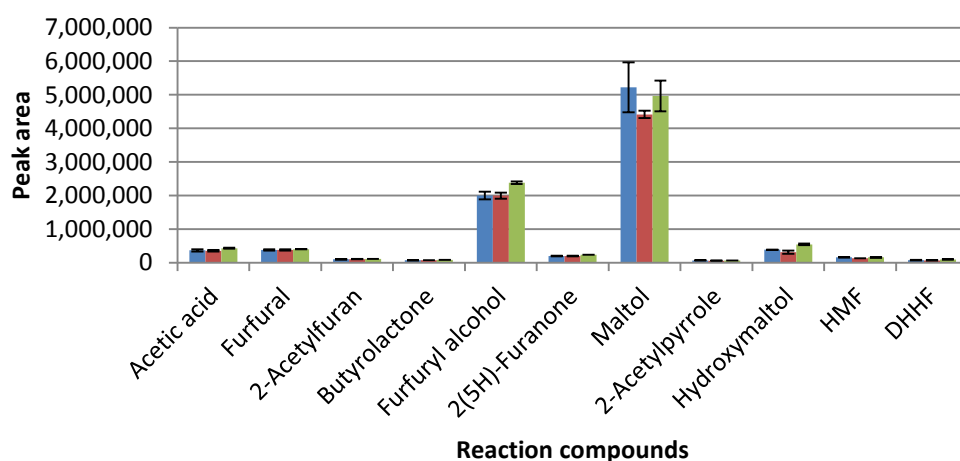


Figure 2.19 The influence of Tween 60 on the analysis of DF300. The DF300 samples have 3% (red), 1.5% (blue) or 0% (green). Tween 60 with error bars representing the standard deviation of replicate samples.

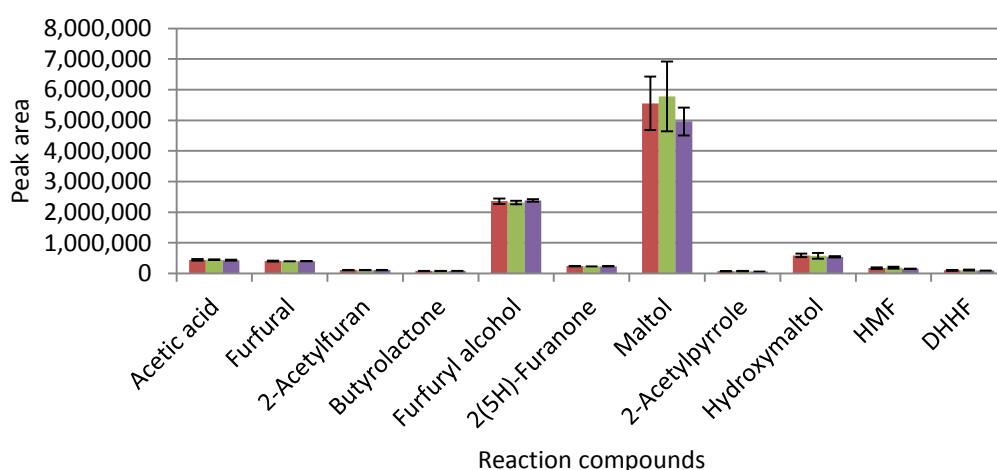


Figure 2.20 The influence of sodium chloride on the analysis of DF300. The samples of DF300 contain 10% (green), 5% (red) and 0% (purple) sodium chloride with error bars representing the standard deviation of replicate samples.

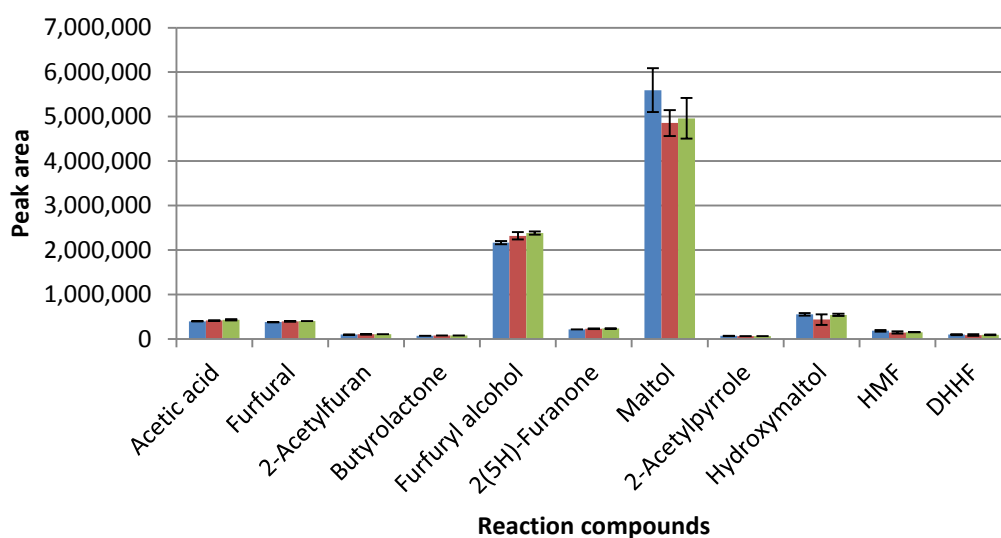


Figure 2.21 The influence of sodium phosphate buffer on the analysis of DF300. The addition of phosphate buffer at the concentration of 0 M (green), 0.2 M (blue) and 1 M (red) in DF300 with the error bars representing the standard deviation of replicate samples.

2.12 Conclusion

A method was successfully developed for the analysis of volatile compounds in ghee involving the use of SPME extraction coupled with GCMS with an error of <20% for replicate analysis of the same sample. This method is robust enough that the addition of emulsifiers and salts to the reaction systems along with changes in the type of fat included will not interfere with the analysis of the volatile compounds. This method will allow any changes to the formation of volatile compounds to be monitored for the assessment of the impact that different structures have on the Maillard and caramelisation reactions in ghee and model systems.

2.13 References

1. Yasuhara, A.; Tanaka, Y.; Hengel, M.; Shibamoto, T., Gas chromatographic investigation of acrylamide formation in browning model systems. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 3999-4003.
2. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C., Evaluation of volatile compounds in different types of ghee using direct injection with gas chromatography-mass spectrometry. *Journal of Dairy Research* **2002**, *69*, 163-171.
3. Nursten, H. E., The flavour of milk and dairy products. 1. Milk of different kinds, milk powder, butter and cream. *International Journal of Dairy Technology* **1997**, *50*, 48-56.
4. Contarini, G.; Povolo, M.; Leardi, R.; Toppino, P. M., Influence of heat treatment on the volatile compounds of milk. *Journal of Agricultural and Food Chemistry* **1997**, *45*, 3171-3177.
5. Philippe, E.; Seuvre, A. M.; Colas, B.; Langendorff, V.; Schippa, C.; Voilley, A., Behavior of flavor compounds in model food systems: a thermodynamic study. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 1393-1398.
6. Mariaca, R.; Bosset, J. O., Instrumental analysis of volatile (flavour) compounds in milk and dairy products. *Lait* **1997**, *77*, 13-40.
7. Fay, L. B.; Brevard, H., Contribution of mass spectrometry to the study of the Maillard reaction in food. *Mass Spectrometry Reviews* **2005**, *24*, 487-507.
8. Lehotay, S. J.; Hajslova, J., Application of gas chromatography in food analysis. *Trac-Trends in Analytical Chemistry* **2002**, *21*, 686-697.
9. Wang, Y. W.; McCaffrey, J.; Norwood, D. L., Recent advances in headspace gas chromatography. *Journal of Liquid Chromatography and Related Technologies* **2008**, *31*, 1823-1851.
10. Dorman, F. L.; Whiting, J. J.; Cochran, J. W.; Gardea-Torresdey, J., Gas chromatography. *Analytical Chemistry* **2010**, *82*, 4775-4785.
11. Yadav, J. S.; Srinivasan, R. A., Advances in ghee flavour research. *Indian Journal of Dairy Science* **1992**, *45*, 338-348.
12. Alonso, L.; Juarez, M.; Abd Rabou, N. S.; El-Shibiny, S., Head space analysis of volatile compounds from buffalo and ewe's Samna (ghee) and butter oil. *Milchwissenschaft - Milk Science International* **2002**, *57*, 76-78.
13. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C.; Przybylski, R., Evaluation of volatile compounds in different types of ghee using headspace with GC/MS. *Milchwissenschaft - Milk Science International* **2003**, *58*, 37-39.
14. Vauthey, S.; Milo, C.; Frossard, P.; Garti, N.; Leser, M. E.; Watzke, H. J., Structured fluids as microreactors for flavor formation by the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2000**, *48*, 4808-4816.
15. Monti, S. M.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A. L.; Fogliano, V., LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *Journal of Agricultural and Food Chemistry* **1999**, *47*, 1506-1513.

16. Rizzi, G. P., Electrochemical study of the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 1728-1731.
17. Rizzi, G. P., Electrochemical aspects of the Maillard reaction and related reactions. *Food Reviews International* **2013**, *29*, 178-191.
18. Jones, A. D.; Tier, C. M.; Wilkins, J. P. G., Analysis of the Maillard reaction products of beta-lactoglobulin and lactose in skimmed milk powder by capillary electrophoresis and electrospray mass spectrometry. *Journal of Chromatography A* **1998**, *822*, 147-154.
19. Liu, X. M.; Metzger, L. E., Application of fluorescence spectroscopy for monitoring changes in nonfat dry milk during storage. *Journal of Dairy Science* **2007**, *90*, 24-37.
20. Andersen, C. M.; Mortensen, G., Fluorescence spectroscopy: A rapid tool for analyzing dairy products. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 720-729.
21. Patton, S., Isolation of maltol from heated skim milk. *Journal of Dairy Science* **1950**, *33*, 102-106.
22. Ajandouz, E. H.; Tchiakpe, L. S.; Dalle Ore, F.; Benajiba, A.; Puigserver, A., Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science* **2001**, *66*, 926-931.
23. Ajandouz, E. H.; Desseaux, V.; Tazi, S.; Puigserver, A., Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry* **2008**, *107*, 1244-1252.
24. Totlani, V. M.; Peterson, D. G., Reactivity of epicatechin in aqueous glycine and glucose Maillard reaction models: Quenching of C-2, C-3, and C-4 sugar fragments. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4130-4135.
25. Zellner, B. D.; Dugo, P.; Dugo, G.; Mondello, L., Gas chromatography-olfactometry in food flavour analysis. *Journal of Chromatography A* **2008**, *1186*, 123-143.
26. Fabre, M.; Aubry, V.; Guichard, E., Comparison of different methods: Static and dynamic headspace and solid-phase microextraction for the measurement of interactions between milk proteins and flavor compounds with an application to emulsions. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 1497-1501.
27. Marsili, R. T.; Miller, N., SPME-GC/MS testing for monitoring off-flavors in processed milk: Application of odor wheel plots. In *Freshness and Shelf Life of Foods*, Cadwallader, K. R.; Weenen, H., Eds. ACS Publications: Washington, DC, United States of America, 2003; Vol. 836, pp 80-94.
28. Balasubramanian, S.; Panigrahi, S., Solid-phase microextraction (SPME) techniques for quality characterization of food products: A review. *Food and Bioprocess Technology* **2011**, *4*, 1-26.
29. Andrewes, P., Changes in Maillard reaction products in ghee during storage. *Food Chemistry* **2012**, *135*, 921-928.
30. DiscoverySciences, Product: GC columns. In www.discoverysciences.com, 2010; pp 216-231.
31. SigmaAldrich, Polyethylene glycol standard. In www.sigmaaldrich.com, 2013.

32. Supelco, GC column selection guide. In www.sigmaaldrich.com, 2013; pp 1-30.
33. Vas, G.; Vekey, K., Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry* **2004**, *39*, 233-254.
34. Kataoka, H.; Lord, H. L.; Pawliszyn, J., Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A* **2000**, *880*, 35-62.
35. Grob, K., Injection technology in capillary GC. *Analytical Chemistry* **1994**, *66*, A1009-A1019.
36. Stashenko, E. E.; Martinez, J. R., Sampling volatile compounds from natural products with headspace/solid-phase micro-extraction. *Journal of Biochemical and Biophysical Methods* **2007**, *70*, 235-242.
37. Miettinen, S. M.; Tuorila, H.; Piironen, V.; Vehkalahti, K.; Hyvonen, L., Effect of emulsion characteristics on the release of aroma as detected by sensory evaluation, static headspace gas chromatography, and electronic nose. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 4232-4239.
38. Kim, T.; Kim, S.; Lee, K., Analysis of furan in heat-processed foods consumed in Korea using solid phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS). *Food Chemistry* **2010**, *123*, 1328-1333.
39. Lee, J. H.; Diono, R.; Kim, G. Y.; Min, D. B., Optimization of solid phase microextraction analysis for the headspace volatile compounds of Parmesan cheese. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 1136-1140.
40. TheGoodScentsCompany, Flavours and fragrances. In <http://www.thegoodscentscompany.com/>, 2012.
41. Leksrisompong, P.; Barbano, D. M.; Foegeding, A. E.; Gerard, P.; Drake, M., The roles of fat and pH on the detection thresholds and coefficients of three compounds: Diacetyl, delta-decalactone and furaneol. *Journal of Sensory Studies* **2010**, *25*, 347-370.

Chapter 3 Ghee

3.1 Introduction

The discussion in this chapter focuses on traditional methods of making ghee, the adaptation of these processes to establish a high throughput production method and the volatile compound profiles of different ghee samples. The volatile compound profile of the ghee samples was investigated using the GCMS method developed previously (chapter 2). External factors leading to changes in the volatile compound profiles such as pH, time and temperature, were explored with respect to their impact on the Maillard reaction. The central hypothesis that the colloidal structures of cream and butter are a key factor in the volatile compound formation is then explored. Such changes in the profile of volatile compounds in ghee offer the potential for manipulating flavour of ghee products.

3.2 Traditional ghee cooking methods

Traditionally, cream (or milk) is fermented by warming to promote growth of intrinsic lactic acid bacteria. Alternatively, cream is inoculated with active cultures. The cultures produce lactic acid, souring and curdling the cream and contributing to the final flavour of the ghee (1-3). There are several artisan methods of making indigenous ghee (figure 3.1) from the soured cream; all of which have a common element of heating to high temperature, 110-140 °C. These different methods all start from fermented milk, cream (often soured to 0.5-1% acidity with lactic acid), butter or a combination of butter and buttermilk (2, 3). After the heat clarification, the remaining product is filtered to remove any remaining solids. Whether the ghee is made by the desi (milk butter) (MB) method, direct cream (DC) method, cream butter (CB) method or the prestratification (PS) method (3) they all have a heat clarification step. The desi or milk butter method (3) involves fermenting the milk for 12-16 hours using lactic acid bacteria and then churning it to make butter (plus buttermilk) before heat clarification to evaporate the water and leave just milk fat (ghee) after filtration to remove non-fat solids (3). The heat clarification step takes place in an open pan at 110-125 °C (4). The direct cream method involves separating the cream from the milk, heating the cream to evaporate the water (110-125 °C) and filtering (4). Cream butter ghee is made with soured cream (0.5-1% acidity value), that is churned to butter before evaporating the water (110-125 °C) and filtering (4). The pre-stratification method involves heating creamery or desi butter to 80 °C and

standing for 30 minutes (3). Once separated the bottom layer can be discarded and the upper layer is heated further to evaporate the water before filtration (4).

An extra ghee preparation method was trialled that was not documented in the literature. Similar to the CB method of ghee manufacture, this method kept the buttermilk produced when the cream was churned to butter. The butter and buttermilk were then cooked together to form the ghee to investigate if it was the structure change (cream versus butter) or different ratios of matrix and starting materials that could lead to the differences in final ghee flavour. The method is referred to as the butter/buttermilk method (B/B method); see figure 3.2.

Samples of ghee made using the direct cream method (chapter 8, section 8.4.1.1), cream butter method (chapter 8, section 8.4.1.2) and butter/buttermilk method (chapter 8, section 8.4.1.3) all exhibited differences in the profile of Maillard compounds. The fermentation step was not carried out when the ghee samples were made in the wok. This step was removed from the wok methods as the contributions to the final flavour from fermentation are not of interest in this study.

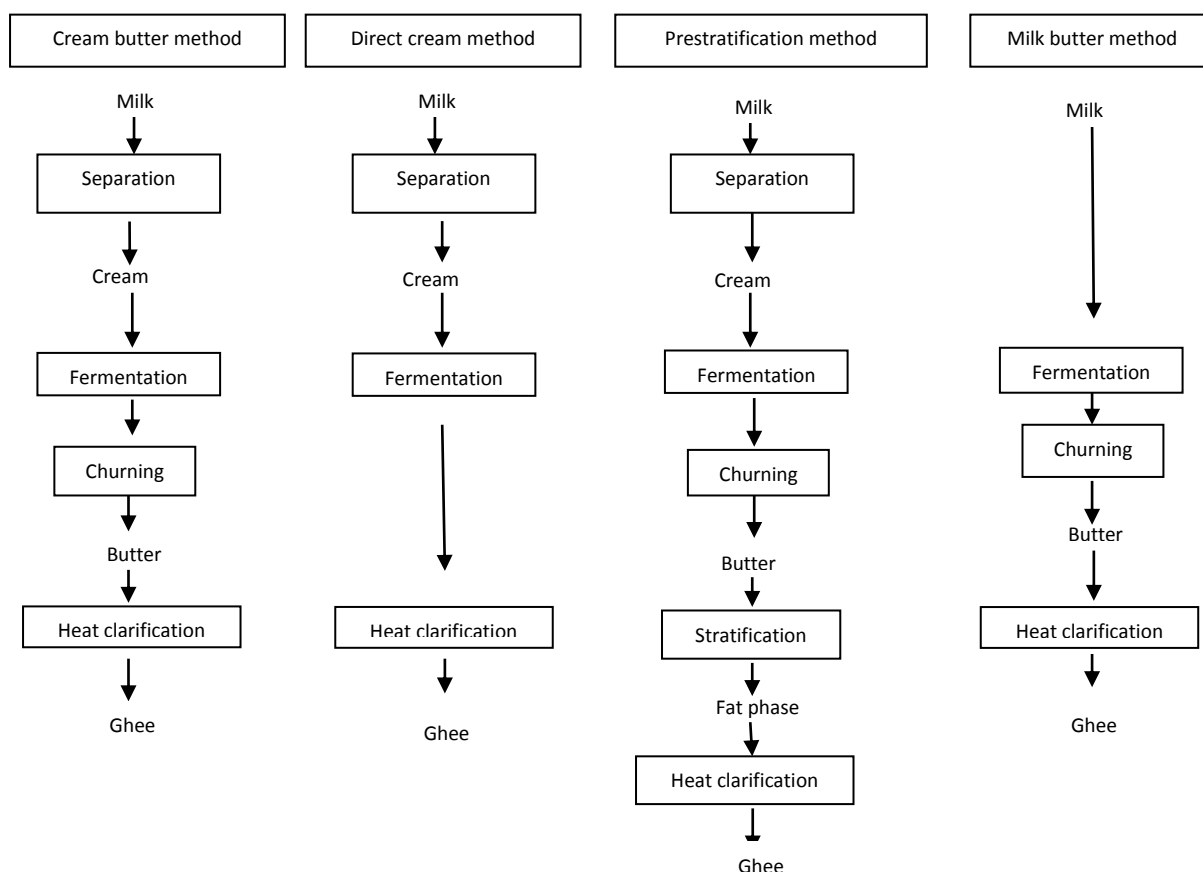


Figure 3.1 Traditional methods of ghee manufacture (3).

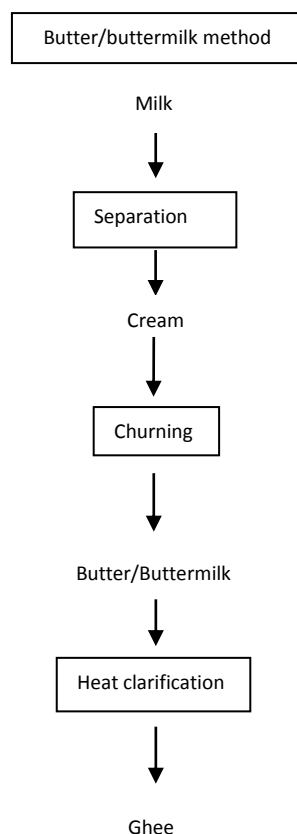


Figure 3.2 Butter/buttermilk method of making ghee, as designed in the laboratory.

The principal components plot uses a correlation matrix based on the peak areas for the selected Maillard compounds as described in section 2.7 (for further details on principal components analysis see appendix C). Flavour contributions due to fat compounds and other volatiles are not included in the analysis of the results. The principal components plot in figure 3.3 has a first component variance of 84.6% and a second component variance of 7.1%. The horizontal shifts account for a large percentage of variation between the different ghee types but also demonstrates the inconsistency in the wok methods (chapter 8, section 8.4.1) from batch to batch. The variation between the samples for each cook type is large relative to the variation between the different cook types. This variation between batches suggests that factors such as the time and temperature of the cooking process are important to the final flavour. Altering the time and temperature could lead to further changes in the final compound profiles. The end point of cooking for each of these batches of ghee was determined using the visual cue of melanoidin colour, which is difficult to pinpoint with accuracy and precision each time. To combat the issue of inconsistency between samples and the time consuming nature of the methods a model cooking method was required (section 3.3).

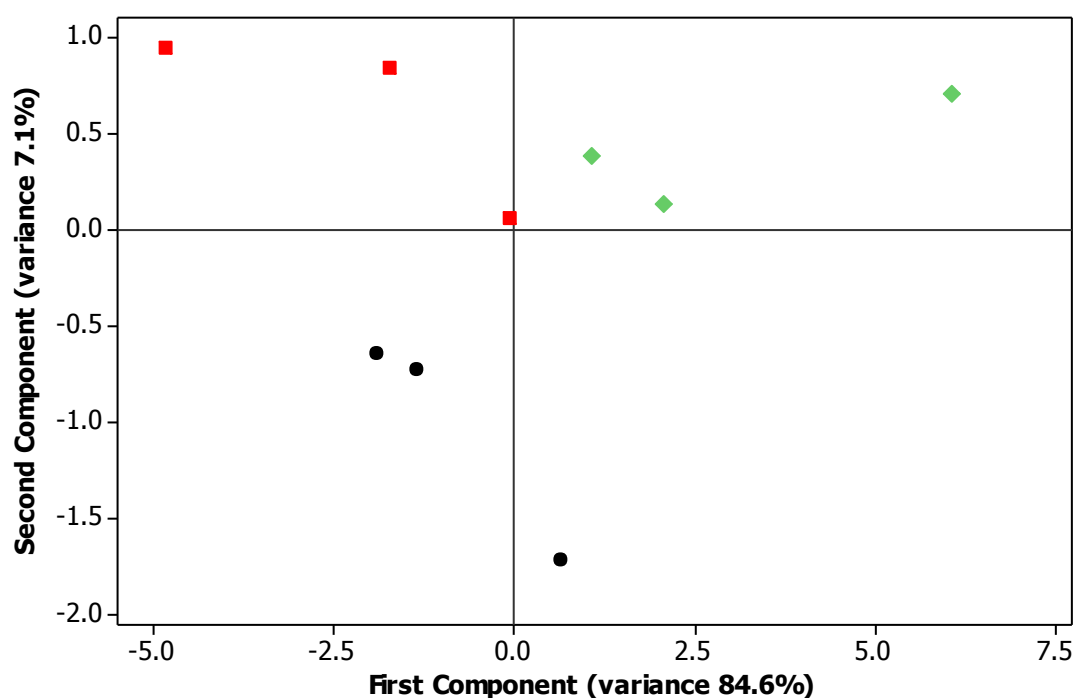


Figure 3.3 Principal components score plot of ghee samples cooked using traditional wok methods. Butter samples are represented in black, cream samples in green and butter/buttermilk samples in red; three batches of each were produced.

3.3 Modelling the cooking of ghee

To effectively study the impact of matrix structure on the formation of volatile compounds in ghee a high throughput and controllable method of cooking small samples was required. By utilising an autosampler agitator attached to the GCMS, the temperature and time of cooking could be controlled along with stirring. Small samples were able to be prepared and cooked using a replicated, high throughput method.

The direct cream and cream butter methods (figure 3.1) were transferred directly to the autosampler (AS) cook setup by placing either cream or butter into the vials. The prestratification method (figure 3.1) was not used due to the extra separation step required which was not practical on a small scale. The butter/buttermilk method (figure 3.2) trialled on the stove top was also applied to the AS cooked ghee.

For all AS methods the filtration step to remove non-fat solids from the ghee was omitted because the presence of the non-fat solids was assumed not to interfere with the equilibrium of volatile

compounds with the SPME fibre. The non-fat solids are melanoidins; these melanoidins are non-volatile large compounds (5, 6) that have been characterised using size exclusion chromatography (7), rheology, thermal and microscopy analysis (8) or solid phase extraction (9) rather than GC.

To establish a set of cooking parameters (time and temperature) a set of experiments were run using the direct cream method (chapter 8, section 8.4.3) to investigate the influence of time and temperature.

3.4 Influence of time and temperature

The time taken to cook ghee is partially related to sample size – the 200 ml sample of cream cooked in the wok takes approximately 60 minutes to cook whereas the smaller 2 ml sample of cream took much less time to go through the same stages of cooking discussed earlier (temperature equilibration, evaporation, further cooking). Cooking times of up to and including 20 minutes were investigated along with a range of temperatures from 110 to 135 °C (1-3, 10-12).

By altering the time and temperature of cooking the ratio of Maillard and caramelisation reactants formed can be shifted. The cooking times and temperatures were varied (chapter 8, section 8.4.3) to gain an understanding of how different compounds act with respect to time and temperature. The accuracy of the temperature regulation in the agitator is ± 2 °C as stated in the user manual. Figure 3.4 shows the differences that occur when the cook temperature is altered by 3 °C. Relative to the differences achieved when the temperature is changed by 10 °C or more (figure 3.5), the changes of ± 3 °C (figure 3.4) lead to relatively minor differences suggesting that any inaccuracy in the temperature control will be insignificant to the final results.

As cooking temperature was increased the yield of Maillard reaction compounds also increased. Temperature can be used to change the volatile compound profile and potentially the flavour of the ghee can be achieved; a lower cooking temperature will provide a ghee containing less of the volatile compounds whereas a higher cooking temperature will provide a ghee containing higher quantities of these compounds. These results were taken from samples of ghee with a cooking time of 20 minutes. When the cooking time is changed, the formation of volatile compounds changes as well (figure 3.6).

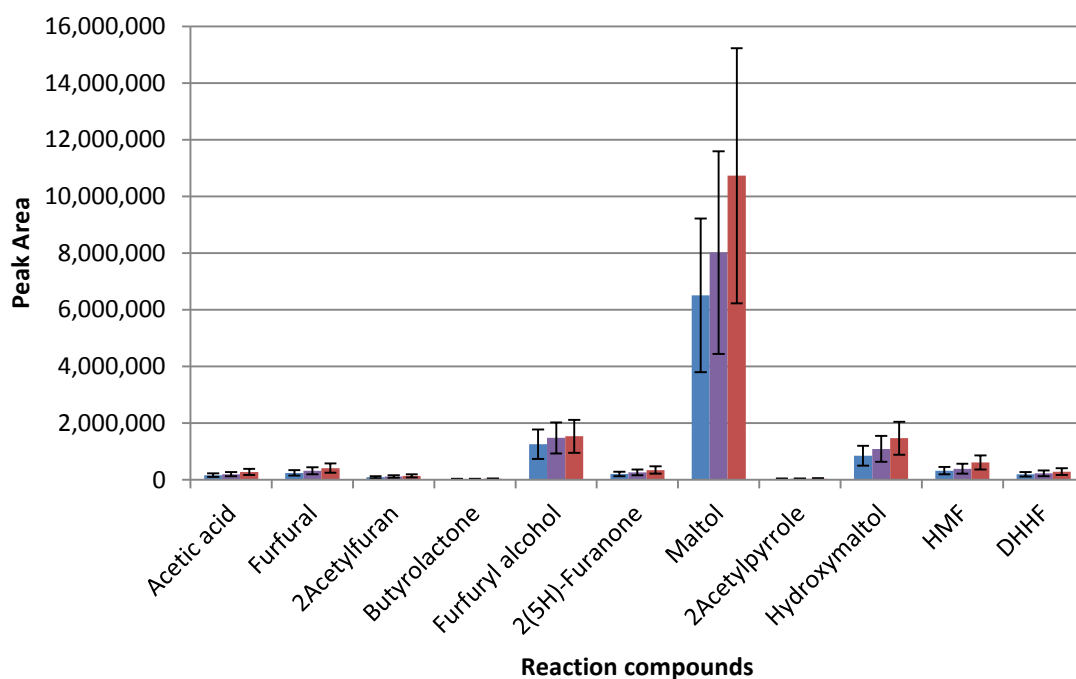


Figure 3.4 Differences generated by a small change in temperature. The temperatures shown on the graph are 132 °C (blue), 135 °C (purple) and 138 °C (red). The error bars represent the standard deviation of duplicate samples.

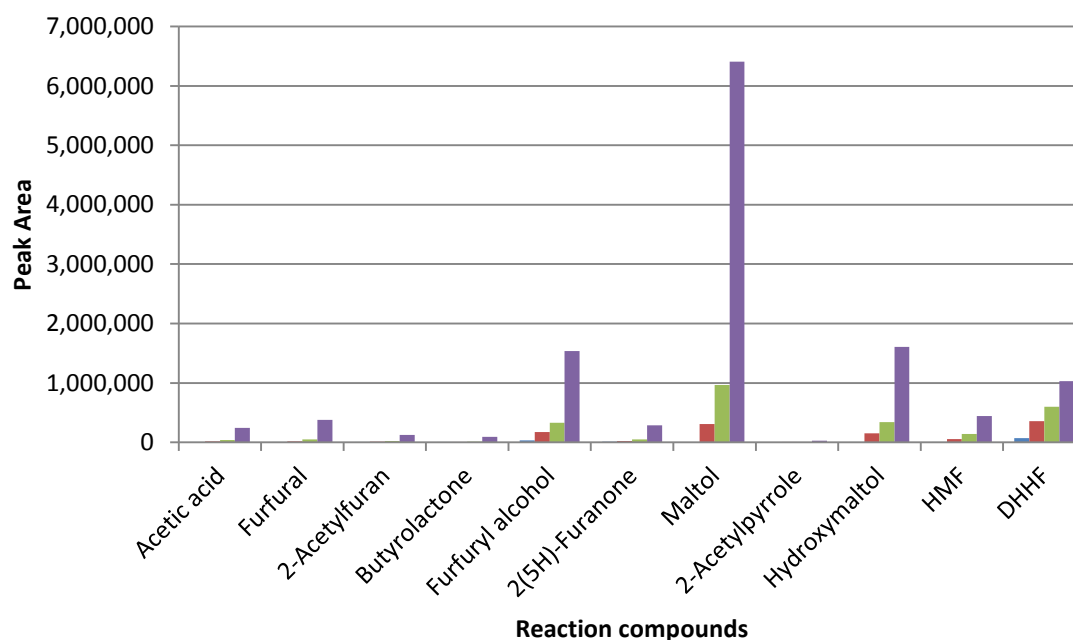


Figure 3.5 Differences generated by large changes in temperature. The temperatures assessed were 110 °C (blue), 120 °C (red), 125 °C (green) and 135 °C (purple). The data in figure 3.5 were not duplicated as they were part of a time series experiment that was only carried out once due to time constraints.

For some of the Maillard compounds it can be seen (figure 3.6) that the effect of temperature and time is greater for some products than for others. There are compounds (furfural, 2(5H)-furanone, maltol) that have little difference in rate or yield at 120-125 °C; however, as soon as the temperature is increased to 135 °C there is an distinct increase in rate overall. When all of the monitored compounds are considered (figure 3.7) an increase in product formation with time was seen at all temperatures tested over the 20 minute time period (figure 3.7). The point at which this rate change occurs differs between the compounds from 12-14 minutes possibly due to change in water activity and the temperature increase that occurs after evaporation. DHHF shows a steady increase over time that increases incrementally with temperature; there is not the large increase in formation that was seen in the other compounds.

After assessing the impact of time and temperature on the volatile compound profile in the ghee samples, a set of cooking parameters was established. Each sample was cooked for 20 minutes at 135 °C to achieve a caramel coloured product containing a range of Maillard reaction compounds.

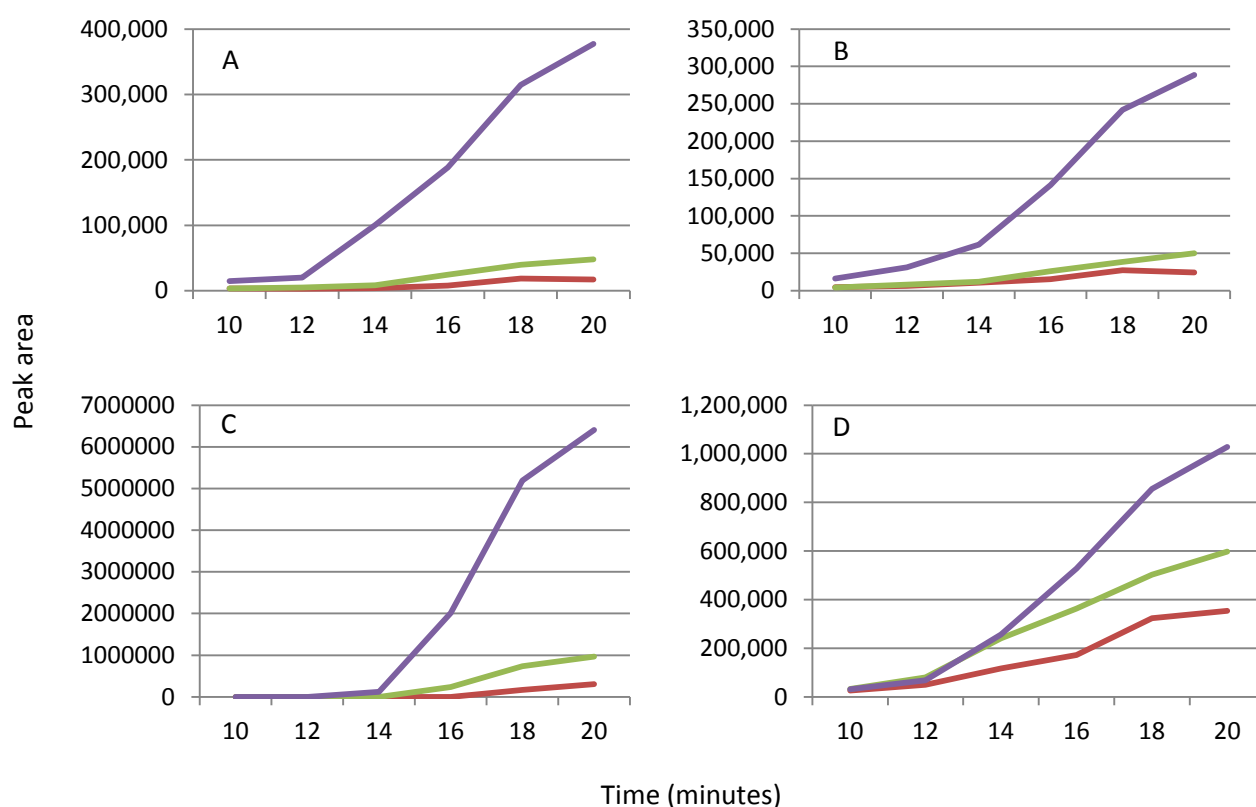


Figure 3.6 Influence of time and temperature on the formation of selected Maillard reaction compounds; A, Furfural; B, 2(5H)-Furanone; C, Maltol; D, DHHF. The temperatures represented on the graphs are 120 °C (red), 125 °C (green) and 135 °C (purple).

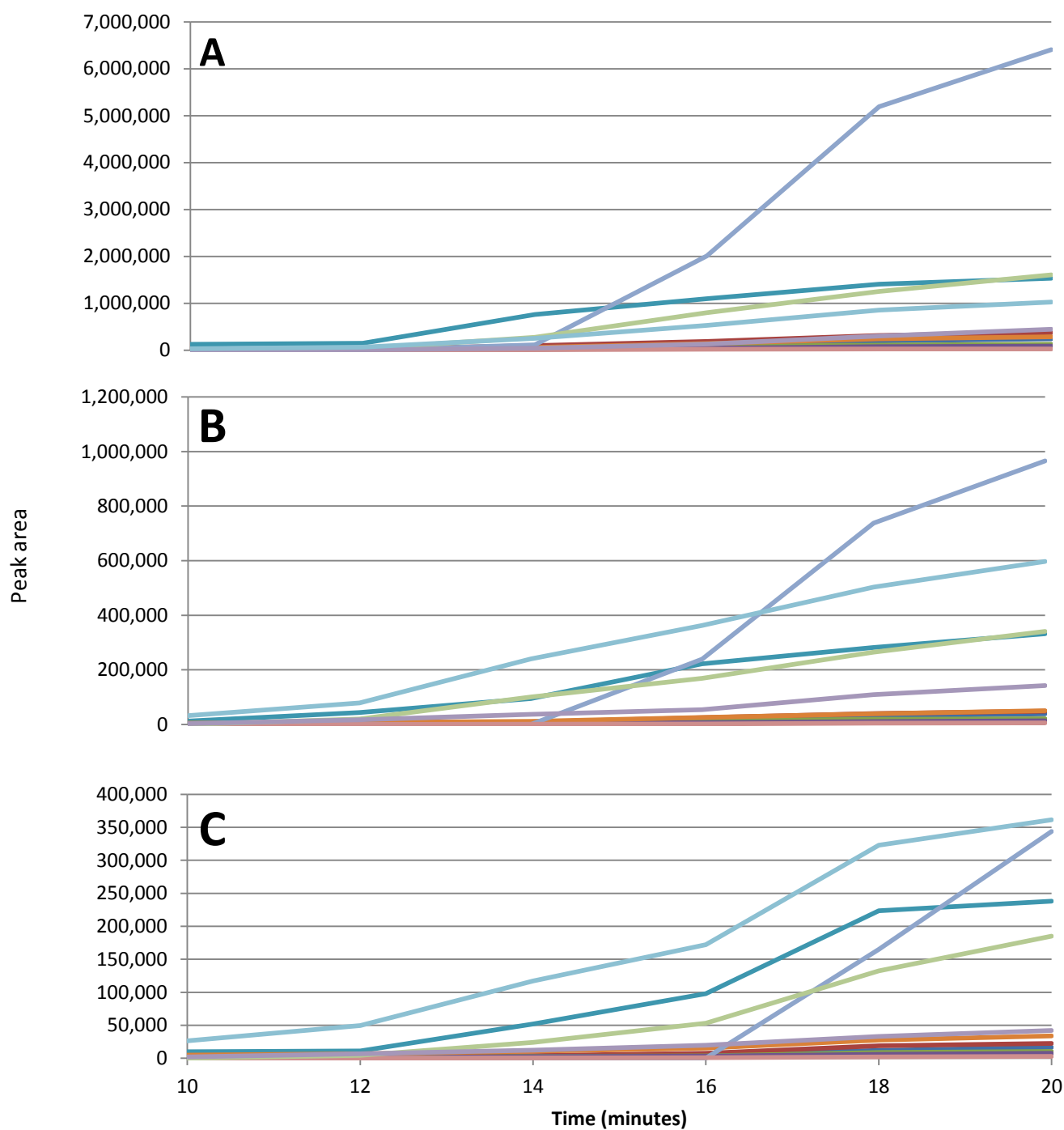
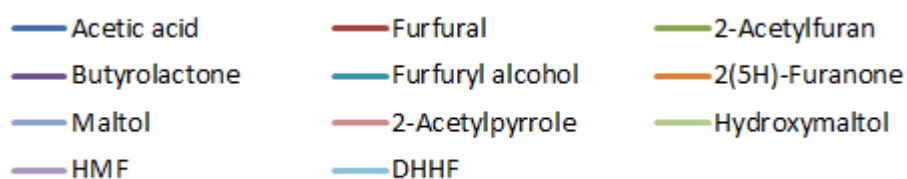


Figure 3.7 Maillard product formation over 10 minutes at; A, 135 °C; B, 125 °C; C, 120 °C.



3.5 Comparing high throughput cooking to wok ghee

The standard cooking parameters adopted for the cooking of ghee in the autosampler were established as 20 minutes cooking time at 135 °C with stirring set to 500 rpm. These cooking parameters were used to cook six replicate samples of each sample: direct cream, cream butter and butter/buttermilk ghee (chapter 8 section 8.4.2). The PCA plot obtained is shown in figure 3.8.

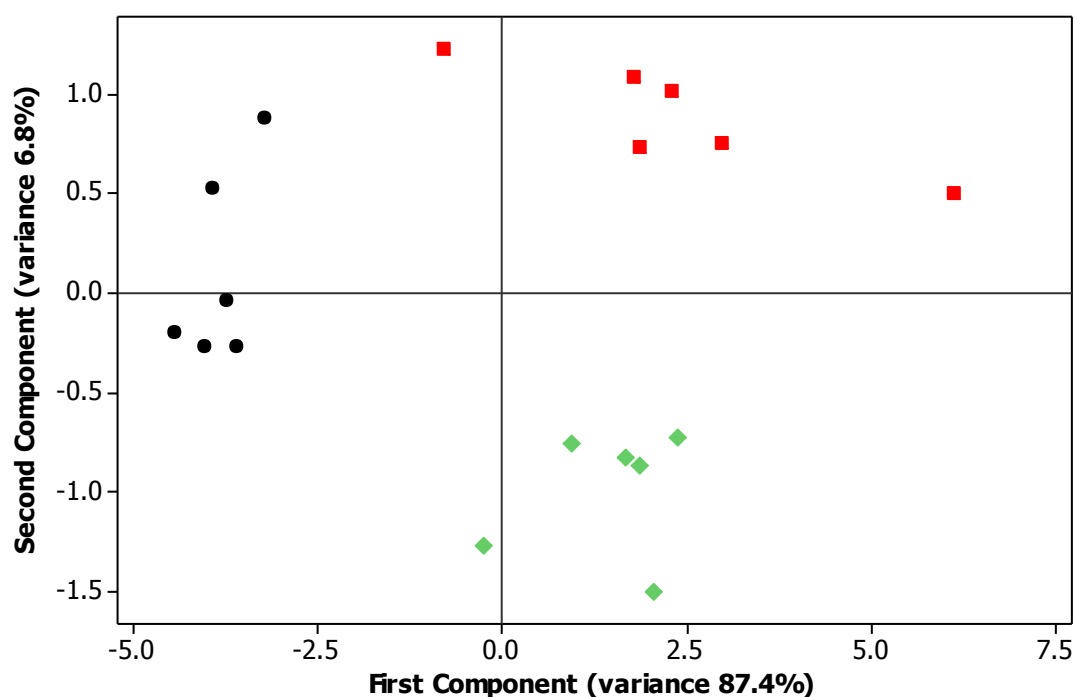


Figure 3.8 PCA score plot of ghee samples cooked using the GC autosampler. Butter samples are shown in black, cream samples in green and butter/buttermilk samples in red.

A comparison of the figure 3.8 PCA plot (correlation matrix) with the plot for the wok ghee (figure 3.3) highlights a similar distribution of sample profiles. The first component explains 87% of the variation with 6.8% explained by the second component. While the percentage in variation is similar between the two figures (3.8 and 3.3), the distribution within the quadrants was different. In figure 3.8 the cream can be seen in the bottom right quadrant with butter and buttermilk positioned in the top half, the distribution in figure 3.3 places cream in the top left quadrant. The different distributions arise from a variation in the ratio of the Maillard compounds.

Ghee cooked in the GC autosampler cannot be expected to mimic ghee cooked in a wok exactly as the conditions of cooking are not able to be 100% replicated. The vials are glass as opposed to the

is cooked using the autosampler. The automated method is not only more consistent, but it is a better model for commercial processes.

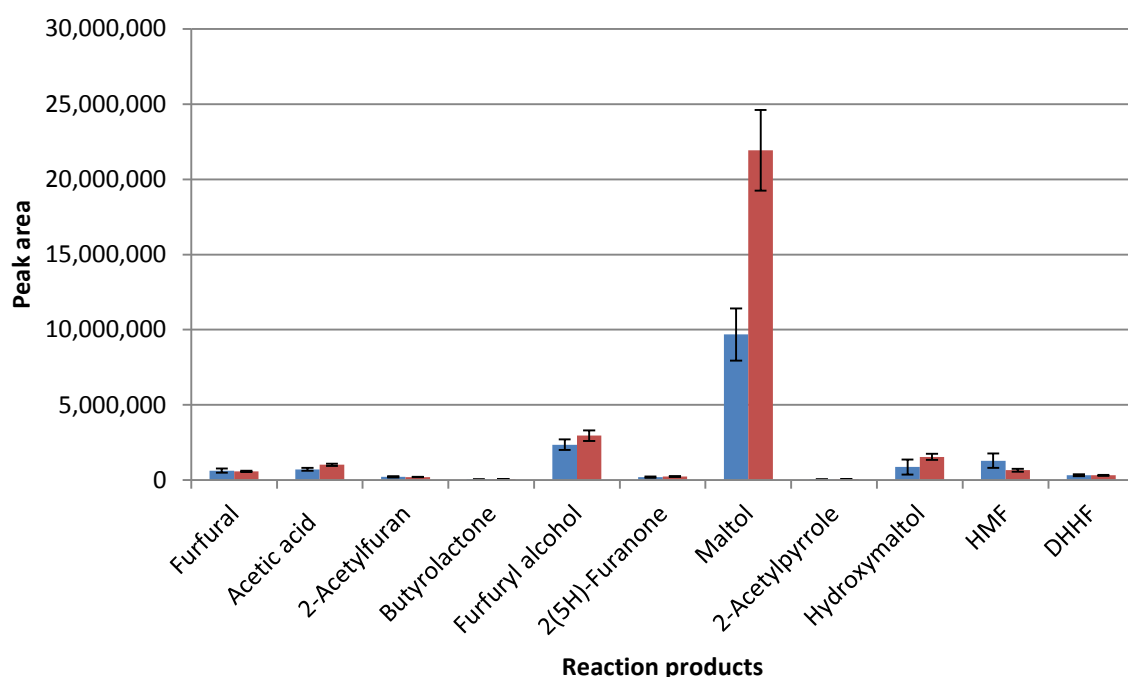


Figure 3.10 Volatile compound profile comparing autosampler and wok cooked cream. Wok cooked cream samples are shown in blue with AS cooked ghee in red. The error bars represent the standard deviation of the six samples.

3.6 Structure of starting materials

As a preliminary experiment, a comparison of different starting materials was used, to see if there was a difference in the volatile compound profile of the ghee. Obviously these starting materials have differences in both chemical composition and physical structure, which does not allow a direct test of the hypothesis. However, the structural differences of each starting material could provide an insight as to why the volatile compound results after cooking are different. Figure 3.11 shows the results from four different ghee samples each cooked in the autosampler (chapter 8 section 8.4.2), for comparison, another sample of model ghee was cooked from a buttermilk sample which contained no fat (chapter 8 section 8.4.2). Starting materials for generating model ghee samples were weighed out such that the amount of aqueous phase would be as consistent as possible across the samples to rule out the possibility of the differences being due to different levels of reactants.

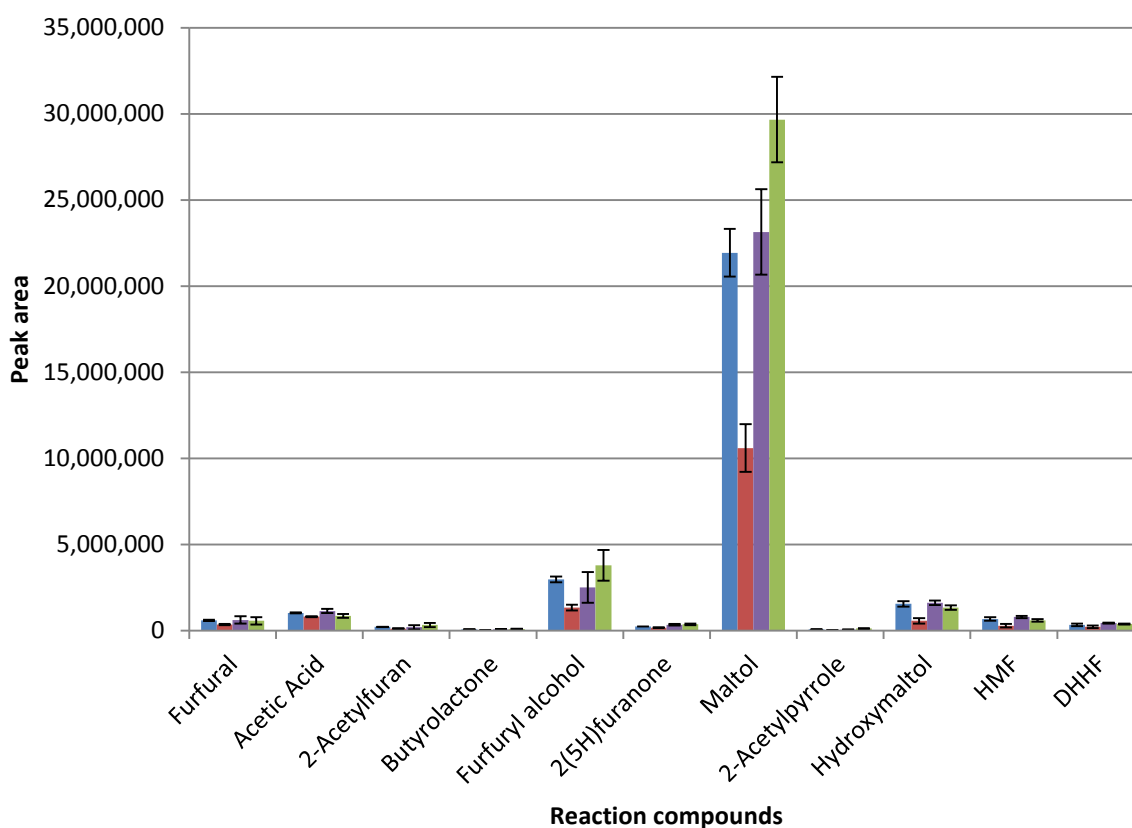


Figure 3.11 Autosampler cooking of different structures of cream. Direct cream method samples are shown in blue, butter samples in red, butter/buttermilk in purple and buttermilk in green. The error bars represent the standard deviation of six samples.

Cream has an oil-in-water emulsion structure. As heat was applied to the system the cream began to simmer and water began to evaporate to create a thick sticky oil in water emulsion. As the water percentage in the matrix decreased further the emulsion underwent a catastrophic transition (13) where the emulsion structure collapsed and did not form a water in oil emulsion. The collapsed emulsion structure which had formed two separate phases of oil and water continued to cook and the remaining water evaporated until only the fat phase remained, along with non-fat solids.

The structure of butter is a water in oil emulsion in which the dispersed water droplets are kinetically trapped and stabilised by the surrounding crystalline fat continuous phase. As butter was heated, the fat phase (which is semi-solid at room temperature) melted and the aqueous phase was released from the fat matrix. The aqueous phase then evaporated without being constrained by a matrix structure.

Along with the structural differences between cream, butter and buttermilk there are also some differences in chemical composition. Cream contains 60% aqueous phase with approximately 3% lactose and 2% protein along with various salts and minerals. Butter contains approximately 20% aqueous phase with the same composition as the aqueous phase of cream – the butter therefore contains only 1/3 of the reactants relative to cream. When the excess buttermilk is retained with the butter the aqueous reactant levels remain the same when compared to cream. Buttermilk that has been removed from cream contains the aqueous components described above but does not contain any of the fat components that could be contributing to the final volatile profile.

To remove the variability associated with chemical composition of the starting materials controlled model systems were needed. The development of these model systems can be found in the next chapter (chapter 4).

3.7 Conclusion

The AS cooking method was developed to cook a ghee sample of small volume with high throughput in an automated system. Differences seen in the samples cooked traditionally in the wok using the various methods could be replicated in the AS samples. The standard method of cooking for the remainder of the study was to use 2 ml cooked in the autosampler agitator for 20 minutes with fast stirring (500 rpm) and a glass bead at 135 °C; any deviations from this standard method are clearly stated.

There were some differences seen between the samples cooked using the same method on both the autosampler and the wok; however, the autosampler cooked samples behaved as expected with respect to pH, time and temperature. In general, the overall Maillard product formation increased as time and temperature were increased between 120 and 135 °C and 10 to 20 minutes, respectively.

There were differences achieved in the volatile compound profile when different starting structures were used; butter versus cream versus butter/buttermilk. The ability to determine which differences are due primarily to the structural differences rather than differences in the chemical composition of the starting ingredients required the use of a model system. The following chapters will continue to explore the impact that matrix structure has on the Maillard and caramelisation reactions. A model system will be discussed in chapter 4 as a simple model for the Maillard and caramelisation reactions in cream.

3.8 References

1. Yadav, J. S.; Srinivasan, R. A., Qualitative and quantitative changes in flavour characteristics of ghee made from ripened cream. *Indian Journal of Dairy Science* **1984**, *37*, 350-356.
2. Ganguli, N. C.; Jain, M. K., Ghee - its chemistry, processing and technology. *Journal of Dairy Science* **1973**, *56*, 19-25.
3. Sserunjogi, M. L.; Abrahamsen, R. K.; Narvhus, J., A review paper: Current knowledge of ghee and related products. *International Dairy Journal* **1998**, *8*, 677-688.
4. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C., Evaluation of volatile compounds in different types of ghee using direct injection with gas chromatography-mass spectrometry. *Journal of Dairy Research* **2002**, *69*, 163-171.
5. Adams, A.; Borrelli, R. C.; Fogliano, V.; De Kimpe, N., Thermal degradation studies of food melanoidins. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4136-4142.
6. Wang, H. Y.; Qian, H.; Yao, W. R., Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry* **2011**, *128*, 573-584.
7. Brudzynski, K.; Miotto, D., Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. *Food Chemistry* **2011**, *127*, 1023-1030.
8. Venir, E.; Pittia, P.; Giavon, S.; Maltini, E., Structure and water relations of melanoidins investigated by thermal, rheological and microscopic analysis. *International Journal of Food Properties* **2009**, *12*, 819-833.
9. Bekedam, E. K.; Roos, E.; Schols, H. A.; van Boekel, M.; Smit, G., Low molecular weight melanoidins in coffee brew. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 4060-4067.
10. Yadav, J. S.; Srinivasan, R. A., Advances in ghee flavour research. *Indian Journal of Dairy Science* **1992**, *45*, 338-348.
11. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C.; Przybylski, R., Evaluation of volatile compounds in different types of ghee using headspace with GC/MS. *Milchwissenschaft - Milk Science International* **2003**, *58*, 37-39.
12. Andrewes, P., Changes in Maillard reaction products in ghee during storage. *Food Chemistry* **2012**, *135*, 921-928.
13. Liu, Y. H.; Friberg, S. E., Perspectives of phase changes and reversibility on a case of emulsion inversion. *Langmuir* **2010**, *26*, 15786-15793.

Chapter 4 An experimental model for the Maillard and caramelisation reactions

4.1 Introduction

This chapter outlines the development and use of reaction models for this thesis. The models were designed to study the Maillard and caramelisation reactions for the purpose of understanding flavour formation in ghee. The reaction models were validated against cream by adjusting the parameters of cooking time, and temperature and starting pH to ensure that the model system behaved in a similar manner to cream when these influential parameters were changed. The addition of phosphates and salt to the model allowed the impact of these components, to be assessed.

A model system was designed based on a very simple view of a cream system. Two simple reaction systems for the Maillard and caramelisation reactions were designed. It can be difficult to separate and distinguish between the caramelisation reaction and the Maillard reaction in any reaction system, particularly in food systems (1, 2). The use of two model reaction systems side by side containing the same quantity of lactose allowed the potential impact of caramelisation pathways to be determined relative to the overall reaction scheme occurring in the Maillard reaction system.

To allow the influence of the chemical composition and matrix to be distinguished, the matrix was broken down into its building blocks, the model system was then built from the bottom up, starting with the simplest matrix and adding components until the model resembled the original matrix. This approach allowed each component of the matrix composition and structure to be investigated and its influence on the reactions determined.

4.2 Modelling the Maillard and caramelisation reaction in cream

Most dairy products including cream and butter contain significant quantities of lactose, a disaccharide of glucose and galactose (3). Lactose and the monosaccharides glucose and galactose are all reducing sugars. Amine groups are available through free amino acids present as well as reactive side chains of lysine residues in proteins. The caramelisation reaction differs from the Maillard reaction in that the reducing sugar (lactose) is degraded in the absence of any amino acids (figure 1.4). In the presence of excess amino groups the Maillard reaction will be favoured. The kinetics of the caramelisation reaction are different from those of the Maillard reaction as the

reaction mechanism that occurs in the absence of the amino groups is different (1, 2) from the mechanisms occurring in the presence of amino groups.

The model reaction systems were developed using D-lactose (3.0% w/v) and L-lysine (2.0% w/v). This equates to concentrations of 87 mL^{-1} (lactose) and 140 mL^{-1} (lysine) and a molar ratio of 1: 1.6; which in turn gives a ratio of lactose: available amino groups of 1:3.2. The lactose content of the model system is a reflection of the lactose content in cream. Lysine was chosen as the amino component of the model system because it is a common and readily available amino acid found in dairy proteins. Lysine also contains a free amino group that is not part of the protein backbone and therefore is free to participate in the Maillard reaction. Lysine was added in excess to explore the limits of the Maillard reaction. Additional lysine could be added to the ghee in a commercial context if excess lysine produced a more favourable flavour profile.

There are other components of cream and dairy products that may also contribute to the Maillard reaction, including phosphates and other salts. These salts can enhance the rate of the Maillard reaction (figure 1.9) and their influence in this model reaction system was explored further in section 4.6. Metals have also been shown to have an impact on the Maillard reaction including calcium, magnesium and sodium (4), all of which are found in dairy products. Sodium was introduced in small amounts as sodium hydroxide to the model reaction systems in order to change the pH. However, the concentration in the sample was less than 0.04 M which is the concentration at which the group I metal ions produced a reliable increase in browning in the ribose/glycine model used in a study by Rizzi (4). The amount of calcium and sodium found in cream is 96 mg/100 ml ($\sim 0.02 \text{ M}$ calcium), 25 – 40 mg/100 ml (0.01-0.02 M sodium) along with magnesium, phosphorus and potassium in quantities ranging from <10 mg/100 ml to 75 mg/100 ml (5) (Fonterra product bulletin, appendix B).

Table 4.1 Amino acids in cream as a percentage calculated from the amino acid profile of milk protein concentrates (Total protein percentage in cream = 2% (Fonterra product bulletin; appendix B)). The fat content of cream was ~40%.

Amino acid	% in cream	g per 100 g of protein
Isoleucine	0.11	5.30
Leucine	0.19	9.60
Lysine	0.17	8.40
Methionine	0.06	2.80
Phenylalanine	0.10	4.90
Threonine	0.09	4.50
Tryptophan	0.03	1.40
Valine	0.13	6.40
Histidine	0.05	2.70
Alanine	0.07	3.30
Arginine	0.74	37.0
Aspartic acid	0.16	7.80
Cysteine/cystine	0.02	1.20
Glutamic acid	0.43	21.6
Glycine	0.04	1.90
Proline	0.20	9.80
Serine	0.11	5.60
Tyrosine	0.12	5.30

4.3 The differences between the Maillard and caramelisation reactions

Using the standard conditions, (chapter 8, section 8.4.2) with a starting pH of 7 (the same pH as fresh cream measured using pH paper (0-14, Merck)) a set of results was obtained for both the caramelisation (figure 4.1) and Maillard reaction (figure 4.2) models that highlights some of the differences in the measured products. Samples were made and cooked in duplicate as per chapter 8, section 8.5.

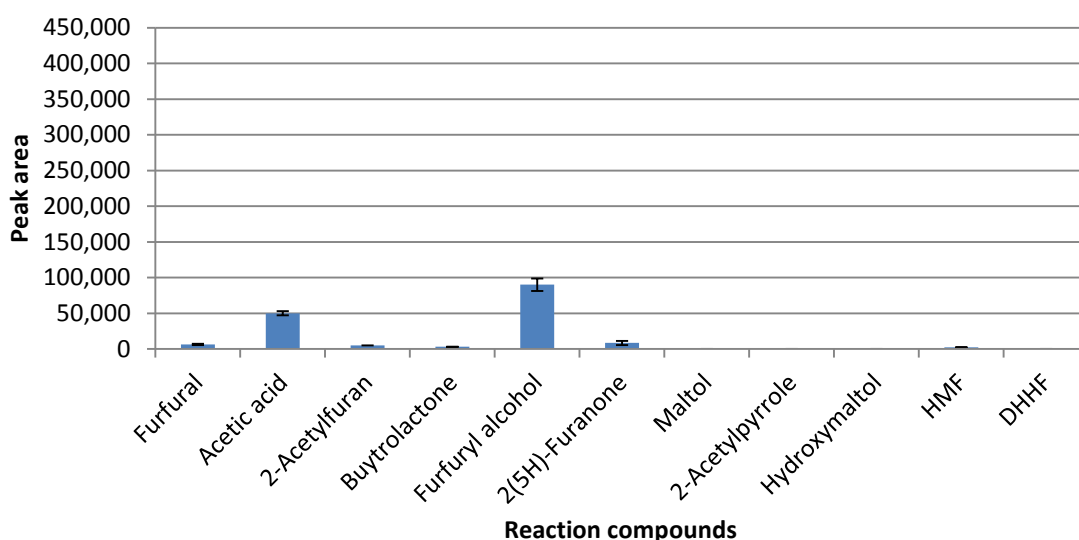


Figure 4.1 Compounds formed via the caramelisation reaction (lactose) at pH 7. Cooking conditions were 135 °C, 20 minutes, 500 rpm stirring. Results are an average of two duplicate cooks of the same aqueous solution as comparison of the caramelisation reaction across multiple experiments can have up to 70% variation in these results. The error bars represent the standard deviation of the duplicate cooked samples for this experiment.

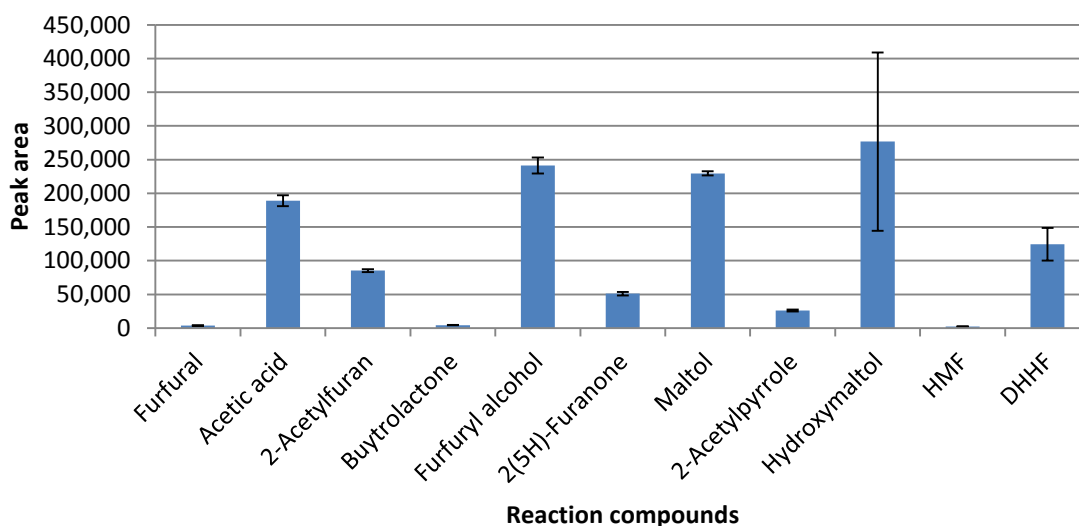


Figure 4.2 Compounds formed via the Maillard reaction (lactose and lysine) at pH 7 in a model system. Cooking conditions were 135 °C, 20 minutes, 500 rpm stirring. Results are an average of two duplicate cooks as comparison of the Maillard reaction across multiple experiments can have up to 70% variation in these results. The error bars represent the standard deviation of the duplicate cooked samples for this experiment.

When cream was cooked (chapter 8, section 8.5.3.1) under the same conditions at pH 7 (pH of fresh cream, no adjustment was made) the same group of compounds were formed although with different ratios (figure 4.3). The pH of fresh cream was measured using 0-14 pH paper.

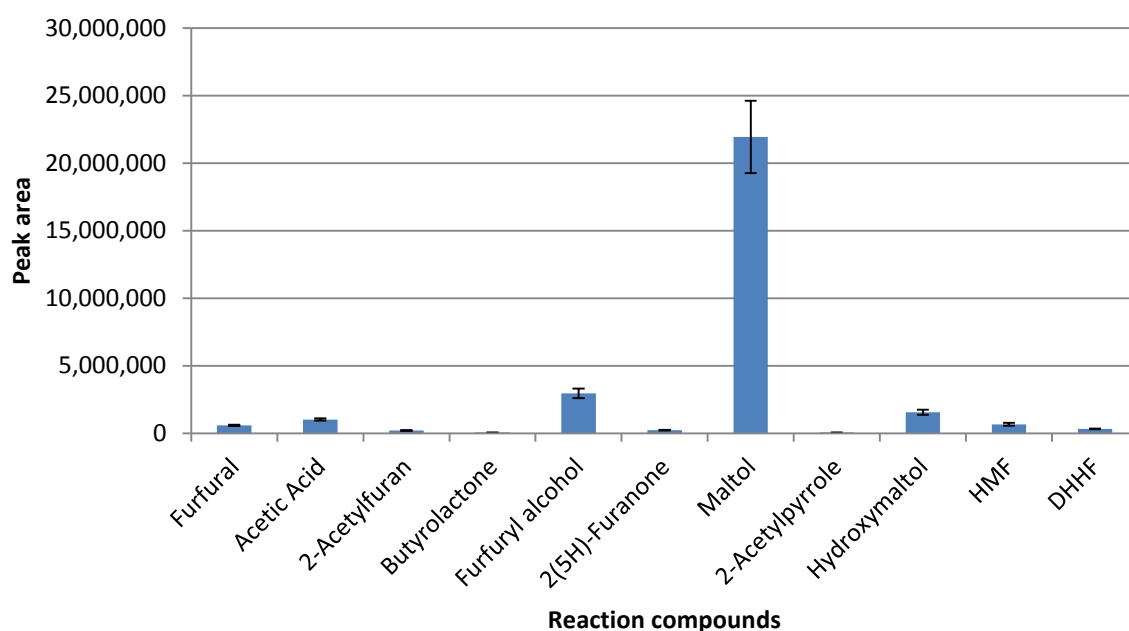


Figure 4.3 Reaction compounds formed via the Maillard and caramelisation reactions in fresh cream. Cooking conditions: 135 °C, 20 minutes, 500 rpm stirring. Error based on the standard deviation of six cooked samples of fresh cream.

The mechanisms of the Maillard and caramelisation reactions of lactose and lysine each have the potential to provide a similar set of volatile compounds (as set out in table 2.2) to those seen in cooked cream and ghee. The Maillard and caramelisation reactions have different initial stages due to the presence of the amino groups in the Maillard reaction (6). The volatile compounds (table 2.2) can be formed by the mechanisms proposed (appendix A) by the Maillard reaction via the Amadori compound (figure 1.2) and subsequent rearrangement (7-11) (figure 1.3), or alternately the compounds can be formed via the caramelisation reaction through a lactose isomerisation pathway (6, 11-14) (figures 1.5 and 1.6). These pathways converge on a set of intermediate compounds: 1-deoxyosone (15-19), 3-deoxyhexosone (6, 19) and lactulose (caramelisation) (6) or aminoreductone (Maillard) (20-22) (figure 4.4) that then react to generate the compounds identified in table 2.2. The only compound that cannot be formed via the caramelisation reaction is 2-acetylpyrrole since the nitrogen from the amino acid is incorporated into the final compound and this is absent in the caramelisation reaction.

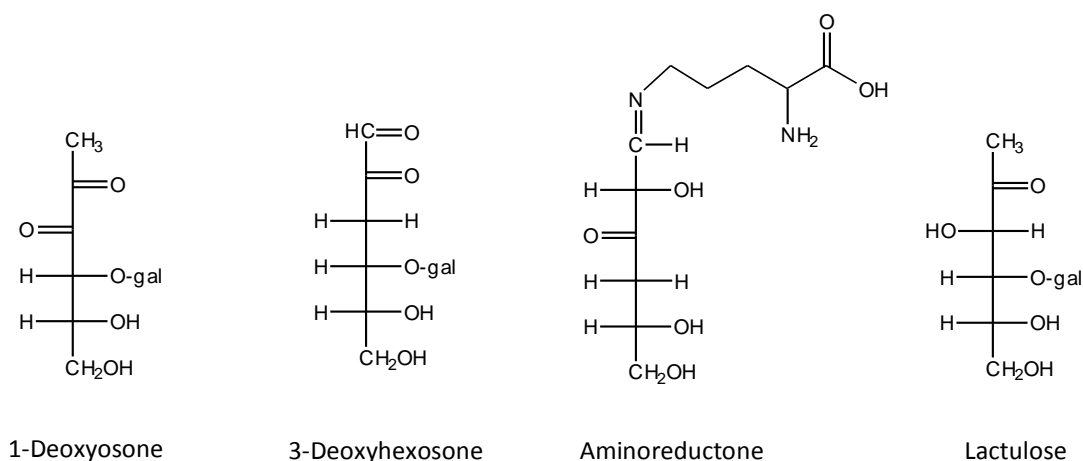


Figure 4.4 Intermediate compounds formed via caramelisation and Maillard reactions en route to volatile flavour compounds based on proposed mechanisms (appendix A).

When the data in figures 4.1, 4.2 and 4.3 were compared for the compounds formed using the caramelisation model, Maillard model and cream it became evident that while the same compounds were formed, they were formed in different ratios, suggesting that some of the reaction pathways are disfavoured in the model systems. Overall the level of compounds found in the cream samples was considerably higher than the level seen in both the caramelisation and Maillard model systems. The ratio of compounds seen in cream was expected to differ from that seen in either of the model systems due to the presence of proteins and free amino acids other than lysine (23). The presence of other amino acids (table 1.1) could provide a range of alternate pathways that may be more or less favoured over those available when only lysine was present in the system leading to different flavours as indicated in table 1.1 where different amino acids were reacted with glucose to achieve different flavours (24). The differences displayed between the model system and cream also highlighted the influence that other parameters such as fat content, emulsion structure and the presence of other components (salts, metals) may have on the formation of the volatile compounds during cooking.

There are similarities between the model Maillard reaction and cream samples; in both acetic acid was detected along with other compounds found along the 2,3-enolisation pathway (figure 1.3). The formation of acetic acid has been used as a marker for the progression of the Maillard reaction (25) in food products such as cereals, where it originates from a 1-deoxyglucosone cleavage (26). This cleavage is along the 2,3-enolisation pathway that occurs after the formation of the Amadori compound and leads to the formation of compounds including maltol, hydroxymaltol and furanones (7) (figure 1.3). In contrast maltol, hydroxymaltol and DHHF were not detected in the caramelisation model in an aqueous matrix (figure 4.5), which would suggest that the mechanisms by which these

compounds are formed occur more readily when an amino acid is present. Furfural, furfuryl alcohol and HMF were formed in both the caramelisation and the Maillard model reactions (figure 4.5). These compounds have been shown to form via an alternate pathway from the Amadori compound rather than the 2,3-enolisation pathway (figure 1.3) in the Maillard reaction (7) and via the caramelisation reaction (11). With the exception of butyrolactone and furfural the Maillard reaction generated a higher yield of all flavour compounds in an aqueous matrix.

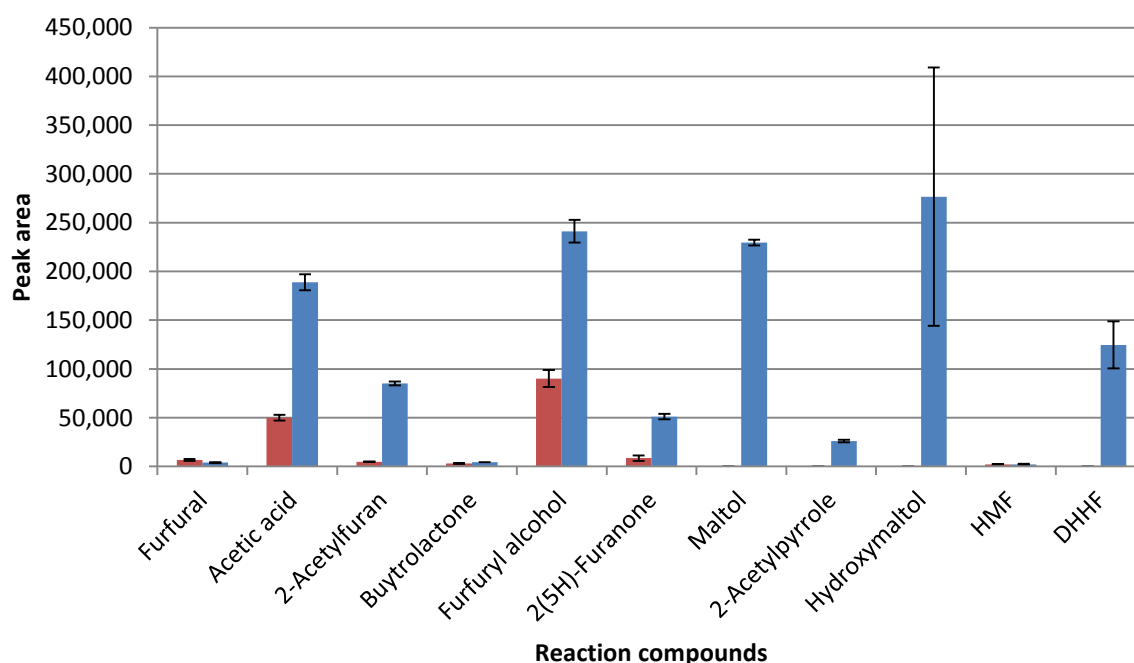


Figure 4.5 Relative amounts of compounds formed in the Maillard model compared to the caramelisation model. Caramelisation reaction samples are represented by the red bars, Maillard reaction samples by the blue bars. The error bars represent the standard deviation of the duplicate cooked samples. The data was taken from figures 4.1 and 4.2.

4.4 Influence of time and temperature

When cooking parameters were altered the amount of flavour compounds in cooked cream generally increased with increasing time and temperature (section 3.4). In cream, both the Maillard and caramelisation reactions can occur at the same time and not all of the protein is available for participation in the Maillard reaction. To validate the model reaction systems against cream a set of samples were cooked against the same time and temperature parameters used to investigate the behaviour of cream.

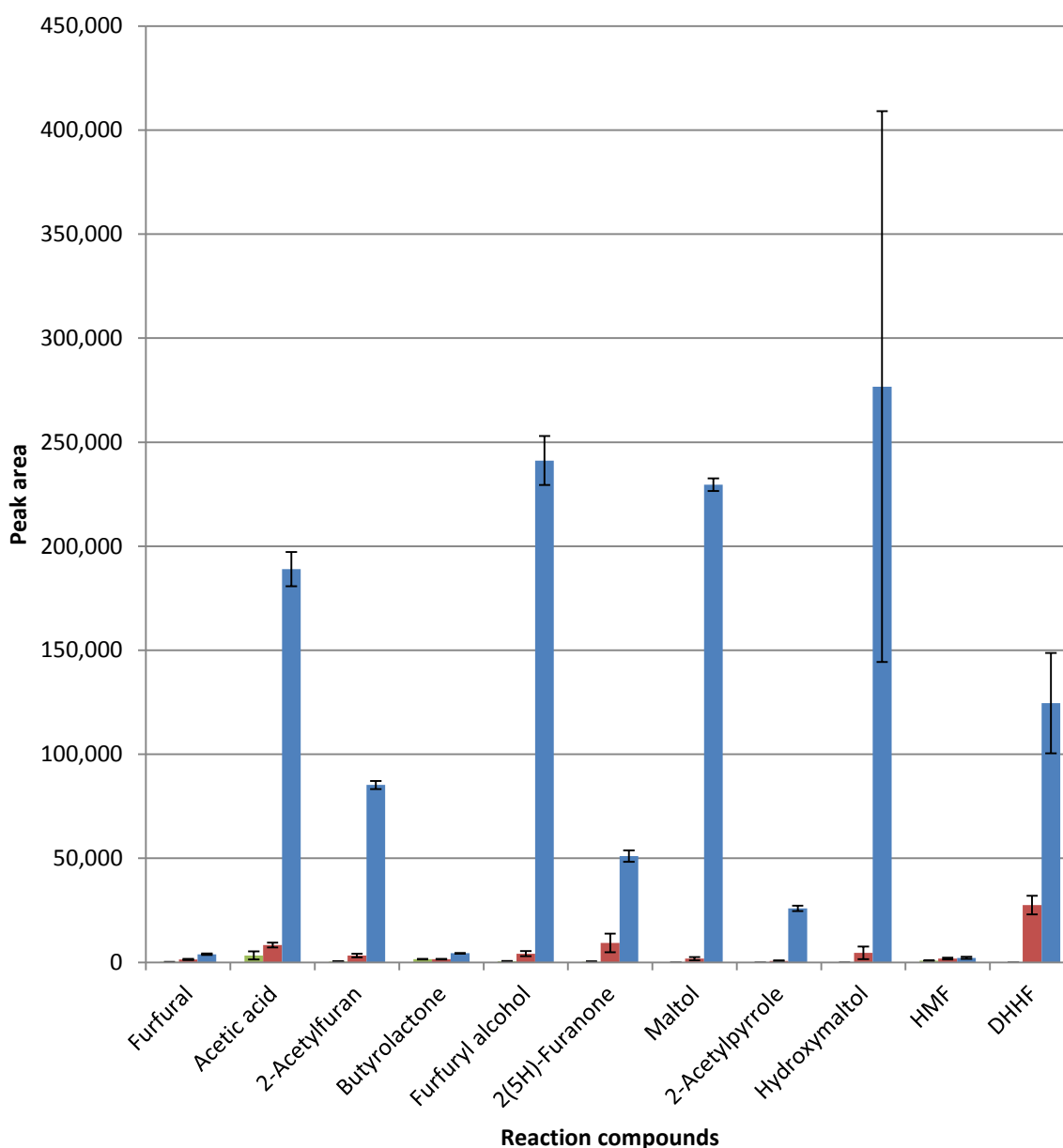


Figure 4.6 Influence of time on the Maillard reaction. Samples of the model aqueous Maillard reaction were cooked at 135 °C for 10 (green), 15 (red) and 20 (blue) minutes. The error bars represent the standard deviation of duplicate cooked samples.

As the time of cooking was lengthened the amount of volatile compound formation increased by a considerable amount (figure 4.6). An increase was also seen when the temperature was increased from 125 to 135 °C (figure 4.7). Similar behaviour with respect to time and temperature was also seen in cream (figure 3.5).

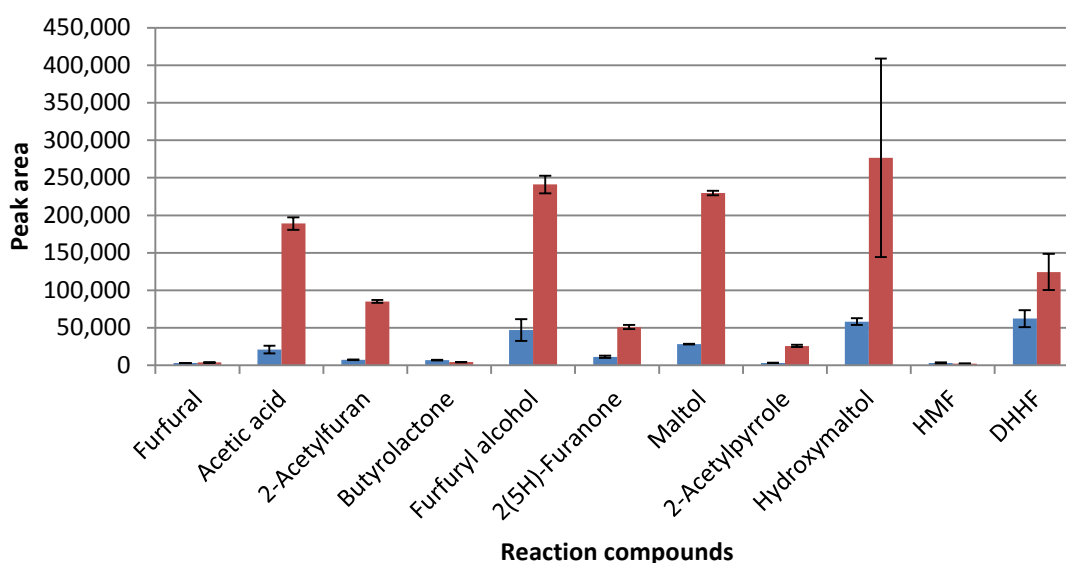


Figure 4.7 Influence of temperature on the Maillard reaction. Samples of the aqueous model Maillard reaction were cooked for 20 minutes at 125 °C (blue) and 135 °C (red). The error bars represent the standard deviation of duplicate samples.

A closer look at the data highlights that the formation of furfural, HMF and butyrolactone was not significantly influenced by temperature and time when compared with the other compounds. The yield of all other compounds significantly increased with longer times and higher temperatures. When these results are compared with the results for the caramelisation there are several points to note. The formation of furfural increased in the caramelisation reaction relative to the Maillard reaction where there appeared to be no significant change with time or temperature in the amount formed. However, butyrolactone appears to decrease with increases in time and temperature during the progress of the caramelisation reaction (figure 4.8 and 4.9). This decrease could suggest that the butyrolactone reacts further as the reaction time increased or that at higher temperatures another compound is forming preferentially. In studies carried out to investigate the formation of Maillard reaction compounds during the breakdown of inulin to make tequila (27), a similar trend was seen during the cooking process whereby the concentration of butyrolactone remained constant and then decreased over time. Along with 2-acetylpyrrole, a compound not expected to be formed in the caramelisation reaction, there are several other compounds that were not detected in this experiment, such as: 2(5H)-furanone (very low detection in figure 4.1 samples), maltol, and DHMF. The absence/low detection of these compounds suggests that other reaction pathways were preferred.

All the other compounds formed during the caramelisation reaction behaved in a similar manner to those formed via the Maillard reaction with respect to time and temperature.

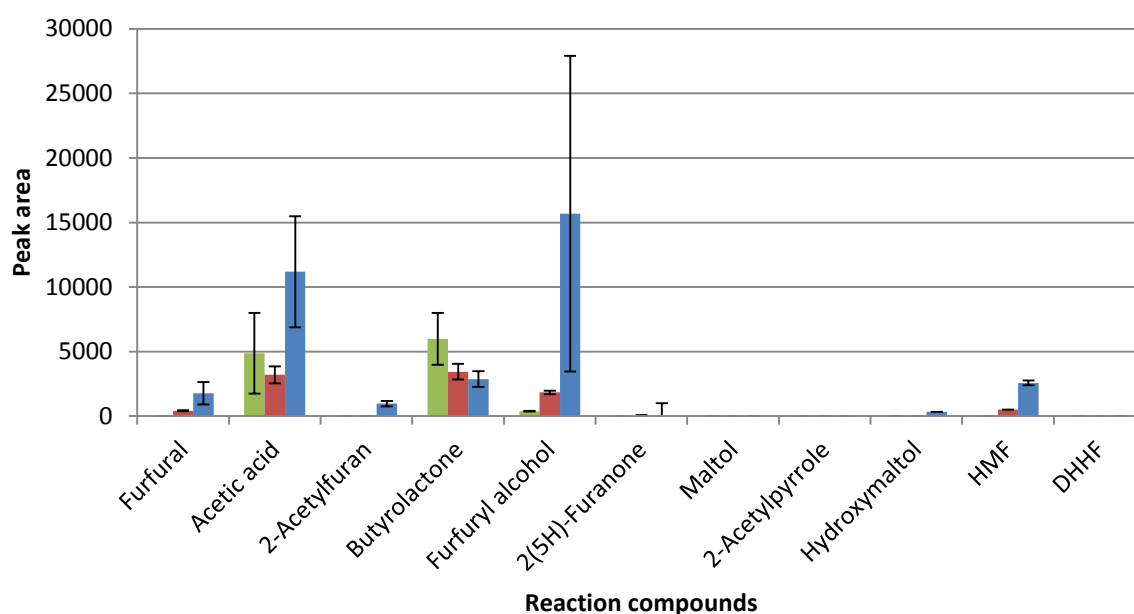


Figure 4.8 The influence of time of the caramelisation reaction in an aqueous matrix. The samples were cooked for 10 (green), 15 (red) and 20 (blue) minutes at 135 °C. The error bars represent the standard deviation of duplicate samples.

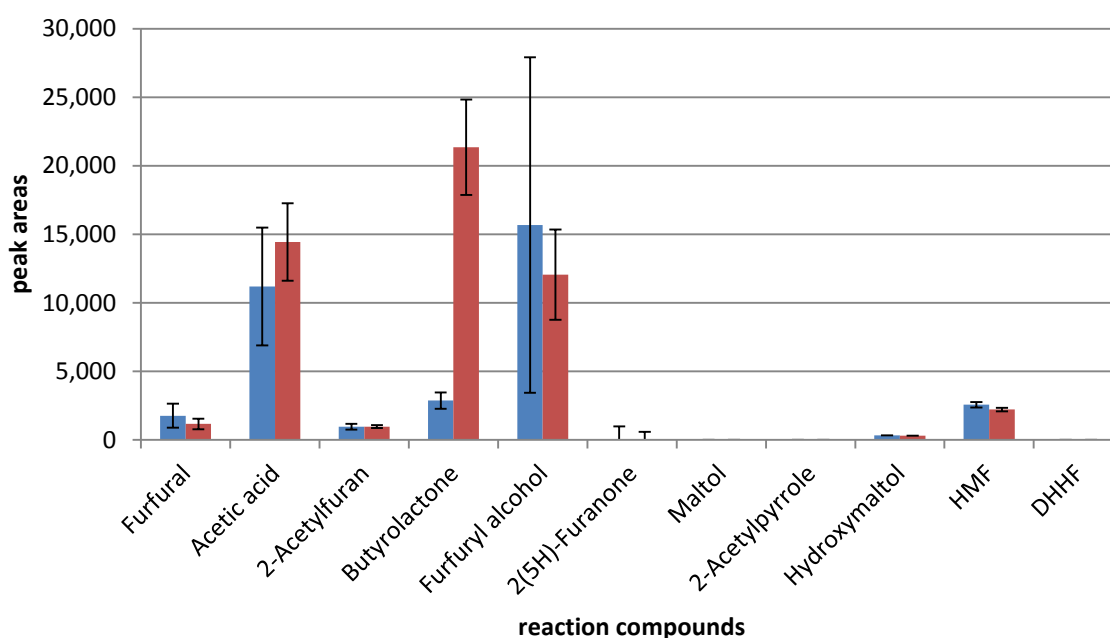


Figure 4.9 The influence of temperature on the caramelisation reaction in an aqueous matrix. The samples were cooked for 20 minutes at 125 °C (red) and 135 °C (blue). The error bars represent the standard deviation of duplicate samples.

Overall, the trend relating to increasing overall volatile compounds with time and temperature was occurring within the Maillard and caramelisation models as was the case during the cooking of the cream samples. A change to time and temperature of cooking alters the overall levels of volatile compounds. The levels of the compounds represent the potential for different levels of flavour and changes in the ratios of compounds to each other can generate changes in the type of flavour. To change these ratios the reaction pathways need to be directed in alternate directions and one method for changing the favoured mechanistic pathway is to change the pH of the reaction.

4.5 Influence of pH on the Maillard and caramelisation reactions

The influence of pH on the Maillard reactions has been previously studied and shown to correlate with the generation of different compounds due to the occurrence of different pathways under different pH conditions (section 1.3.1) (1-3, 28-30). Based on this literature it was hypothesised that the formation of volatile compounds in ghee could be altered by using cream that had been adjusted to different pH values (chapter 8, section 8.5.3.1). The pH of the system changes as the reaction progresses and so it is difficult to control the reaction pH without the addition of a buffering system to maintain a constant pH; however, the addition of these buffers can also impact on the reaction (section 1.3.1).

Even with the buffering capacity of cream (31) there was decrease in measured pH as the reaction progressed. This is in part due to the formation of acidic compounds (32, 33). This pH decrease was seen across all of the cream samples (chapter 8, section 8.5.2) regardless of the starting pH, the final pH for all samples was 4-5. The decrease of pH observed in the cream solution (figure 4.10) during cooking was also observed in the caramelisation experiments, and has been documented as occurring during caramelisation leading to lactulose degradation into fructose and galactose (12). The model reaction system samples all followed the same pH trend (figure 4.11).

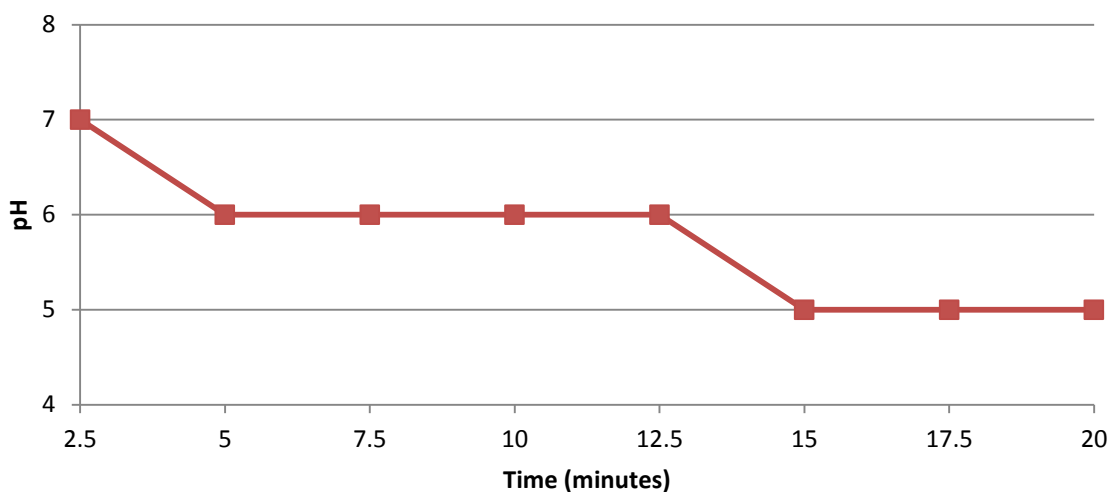


Figure 4.10 Change in pH of cream at 2.5 minute intervals as it was cooked at 135 °C for 20 minutes. The pH was measured using 0-14 pH paper.

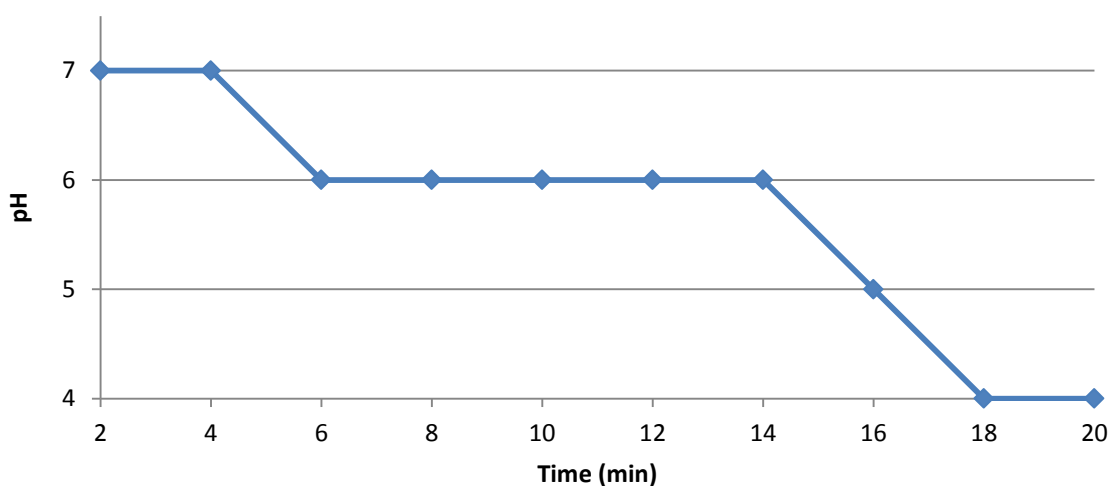


Figure 4.11 Change in pH at 2 minute time intervals of the aqueous Maillard reaction model as it was cooked at 135 °C for 20 minutes. The pH was measured using 0-14 pH paper.

By adjusting the starting pH of the system the rate of change in reaction pH over time will be altered leading to differing amounts of time at which any one mechanistic pathway is favoured for the formation of volatile compounds. As such, different ratios of volatile compounds were expected when the pH of cream and the model systems was adjusted prior to cooking. There was a distinct change in the volatile compound profile of the ghee. The mechanism shown in figure 1.3 predicted

that furanone would decrease with decreasing pH and that HMF would increase along with furfural due to the preference for 2,3-enolisation mechanisms at high pH and 1,2-enolisation mechanisms at lower pH (1, 2, 19, 30). Furfural formation did not appear to follow a trend as the starting pH was altered despite the mechanisms by which it is formed being reported (3) to require acidic conditions.

The influence of pH on the Maillard reaction products is due to the different reaction pathways from the Amadori compound (figure 1.3). The different reaction mechanisms are pH dependent with the 2,3-enolisation pathway dominant at high pH values and the 1,2-enolisation pathway dominant at lower pH values. The proposed mechanism for the formation of HMF indicate that acidic conditions are required (figure 1.3) and its formation occurs along the 1,2-enolisation pathways whereas the furanones are formed along the 2,3-enolisation pathway (figure 1.3) via the deoxyhexosone compound (figure 4.4).

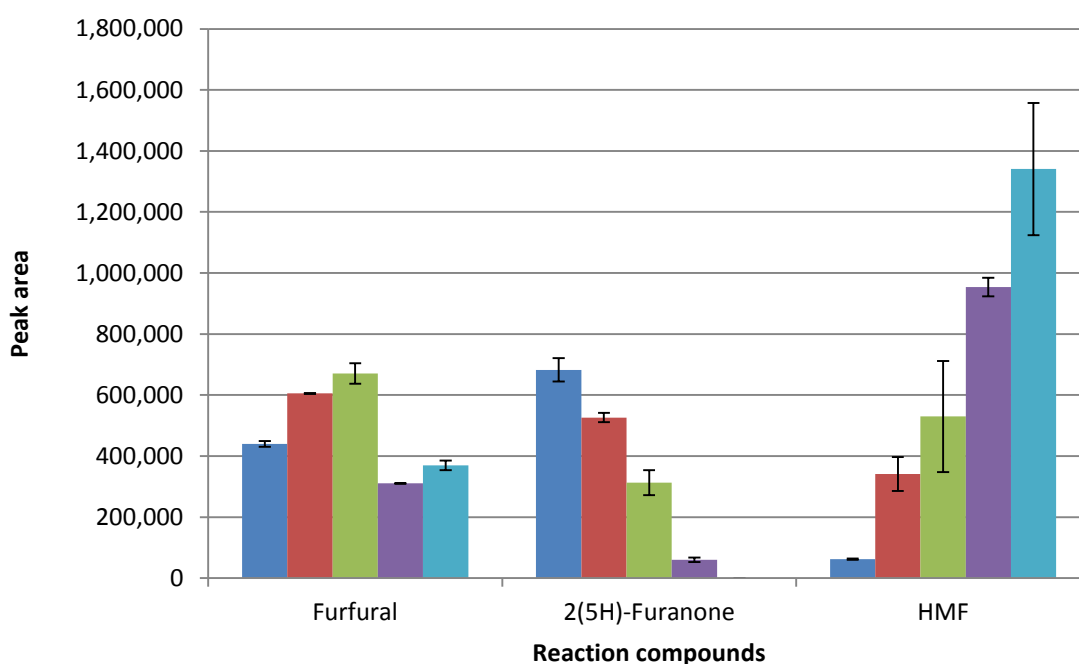


Figure 4.12 The change in formation of furfural, 2(5H)-furanone and HMF in cooked cream at different pH values. Error bars represent the standard deviation of duplicate cooked cream samples.

■ pH 11 ■ pH 9 ■ pH 7 ■ pH 5 ■ pH 3

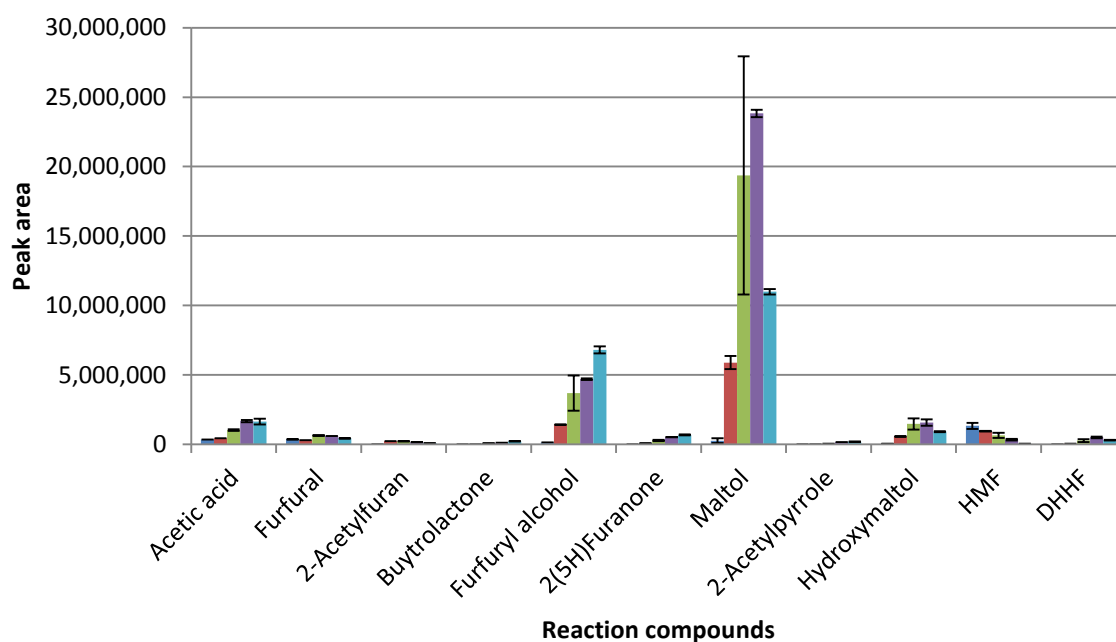


Figure 4.13 Influence of different starting pH on volatile compound formation in cream cooked at 135 °C for 20 minutes. Error bars represent the standard deviation of duplicate cooked cream samples. ■ pH 3 ■ pH 5 ■ pH 7 ■ pH 9 ■ pH 11

The effect of pH on the overall volatile compound formation in cream can be seen in figure 4.13. Each compound has an independent optimum pH of formation meaning that different compound ratios can be generated through a simple pH change. At an initial pH of 11 furfuryl alcohol, 2(5H)-furanone, butyrolactone and acetic acid are formed at their highest yields, these yields then decrease as the starting pH is decreased. Other compounds, such as maltol and the related compound hydroxymaltol, had an optimal yield at pH 9 with HMF favoured at acidic pH. The same effect was seen in the model reaction systems (chapter 8, section 8.5.3) when the starting pH was adjusted. Different changes in individual compounds were seen in the caramelisation reaction and the Maillard reaction models.

When the caramelisation reaction was carried out at an initial pH of 11 there was a significant increase in the formation of most of the compounds, particularly furfuryl alcohol (figure 4.14). Whilst it is difficult to directly compare the results from the caramelisation model with those from cream (due to the multiple reactions that occur in cream) the increase in furfuryl alcohol at pH 11 was in line with an increase seen in cream.

Caramelisation of lactose can occur through multiple pathways via the isomer lactulose (11, 12, 34, 35). Isomerisation of lactose to lactulose (figure 1.5) occurs as a series of reversible reactions. The position of the equilibrium and therefore the balance of isomers can be pushed towards lactulose by using sodium hydroxide at pH 11 and by heating the solution (12). Given the impact seen when the pH was adjusted to pH 11 prior to cooking it was likely this isomerisation to lactulose was occurring at an increased rate in the model system.

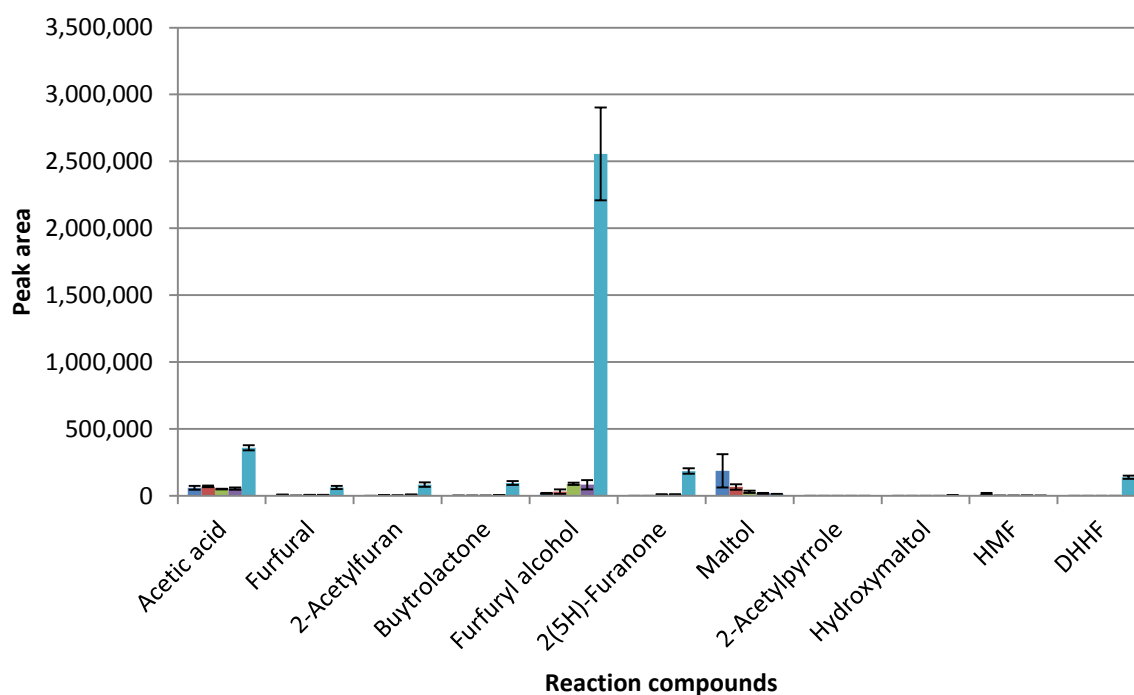


Figure 4.14 Variation in product formation for the caramelisation reaction with a change in the initial pH. Error bars represent the standard deviation of duplicate cooked samples

■ pH 3 ■ pH 5 ■ pH 7 ■ pH 9 ■ pH 11

A closer look at the results obtained when the initial pH of the aqueous caramelisation reaction is changed (figures 4.14 and 4.15) show that the formation of furfural exhibits only a little variation as the pH was varied while HMF formation was increased at low pH and 2(5H)-furanone increased with increasing pH.

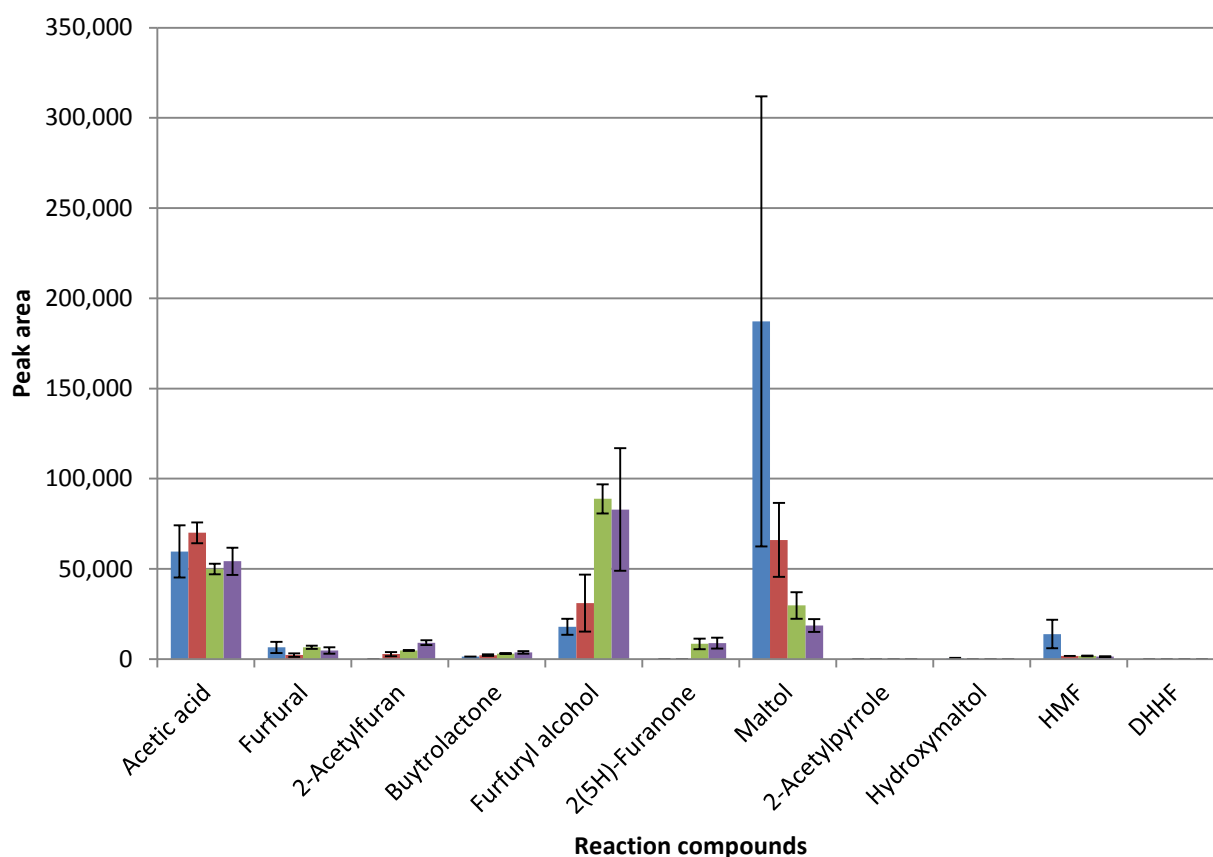


Figure 4.15 Changes to the caramelisation reaction with pH (pH 11 results have been removed for clarity). Error bars represent the standard deviation of duplicate cooked samples.

■ pH 3 ■ pH 5 ■ pH 7 ■ pH 9

The increase in the formation of volatile compounds at high pH can be attributed to the protonation state of the amine groups on the lysine residue (figure 1.8). The initial stages of the Maillard reaction up to the formation of the Amadori compound occur at a slower rate when the amine groups are protonated under acidic conditions. Adjusting the reaction solutions to a higher pH resulted in a faster reaction that yielded higher levels of several key compounds including acetic acid. Based on these results (figure 4.16) it would appear that an initial reaction pH of 9 would yield the greatest level of product formation for the Maillard reaction model. The 2,3-enolisation pathway (figure 1.3) of the Maillard reaction has been shown to be favoured at basic rather than acidic pH. As can be seen in figure 4.16 compounds such as maltol, hydroxymaltol, DHMF and furanone that are found along the 2,3-enolisation pathway (figure 1.3) were prominent at pH values above 7. An increase in acetic acid, a by-product of this pathway also increased at higher pH values, contributing to the overall decrease in pH (figure 4.11).

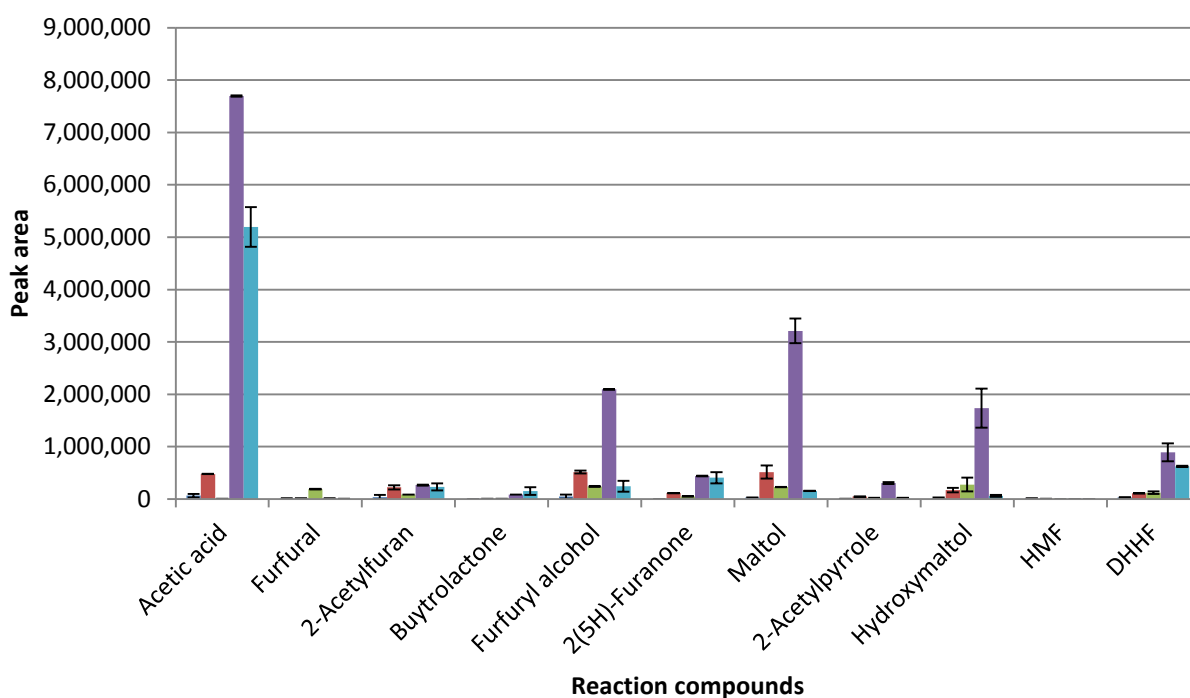


Figure 4.16 Influence of pH on the Maillard reaction model. The error bars represent the standard deviation of duplicate samples. ■ pH 3 ■ pH 5 ■ pH 7 ■ pH 9 ■ pH 11

4.6 Other components of cream that influence the Maillard reaction

As previously indicated there are many components in cream and related dairy products other than lactose and lysine including phosphates and other salts (31). These compounds are also able to influence the Maillard and caramelisation reactions (36). The reaction models contained only the reactants, whereas cream on the other hand contains a myriad of buffers and salts each of which can buffer the pH (31) and catalyse the reaction (37) as shown in figure 1.10.

Since the decrease of pH over the course the Maillard reaction can slow the initial reaction rate, the addition of a buffer can stop this pH decrease (37) depending on the reaction parameters. Studies carried out at 37 °C showed no change in pH over the course of the reaction when a pH 7 phosphate buffer (0.02 - 0.5 M) was added to the reaction system (37). This is contrary to what happened in cream which contains its own buffering capacity which has an optimum capacity at pH 3-6 (31) and has a final pH of ~pH 5 when cooked for 20 min at 135 °C. The addition of buffers and salts to the model system was used to gain an understanding of the impact the buffering species present in cream could have on the Maillard and caramelisation reactions. A 0.2 M phosphate buffer was added to the model systems along with 5% NaCl as per chapter 8, section 8.5.4/8.5.5. At a

concentration of 0.2 M the phosphate buffer was roughly 50% of the maximum buffer concentration used by Bell (1997) (37) and exceeded the concentration of lysine in the system. The 5% NaCl provided 0.3 M of sodium to the reaction system which exceeds the amounts used in the study by Rizzi (2008) (4). At these concentrations any influence of the phosphate or sodium addition should be detected in changes to volatile compound formation. Each sample was cooked and analysed once to provide an indication of the potential impact that the addition of sodium chloride and phosphate buffer may have on the reactions.

There is an obvious impact on both the Maillard (figure 4.17) and the caramelisation (figure 4.18) when the phosphate buffers are added. Any phosphates present in cream can therefore be expected to have a large impact on the overall progress of each reaction either due to the buffering provided or the interaction of the phosphate groups (figure 1.9).

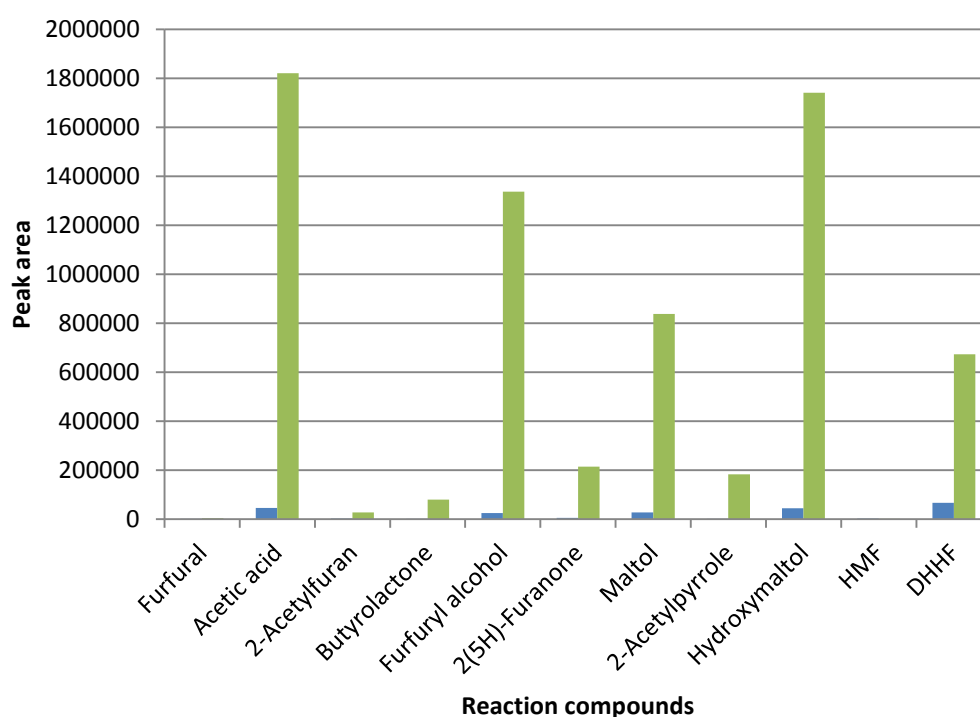


Figure 4.17 Impact of phosphate buffer addition to the Maillard reaction. Maillard reaction samples containing 0.2 M phosphate buffer (pH 7) shown by the green bars and samples without phosphate buffer shown by the blue bars. This experiment was not replicated.

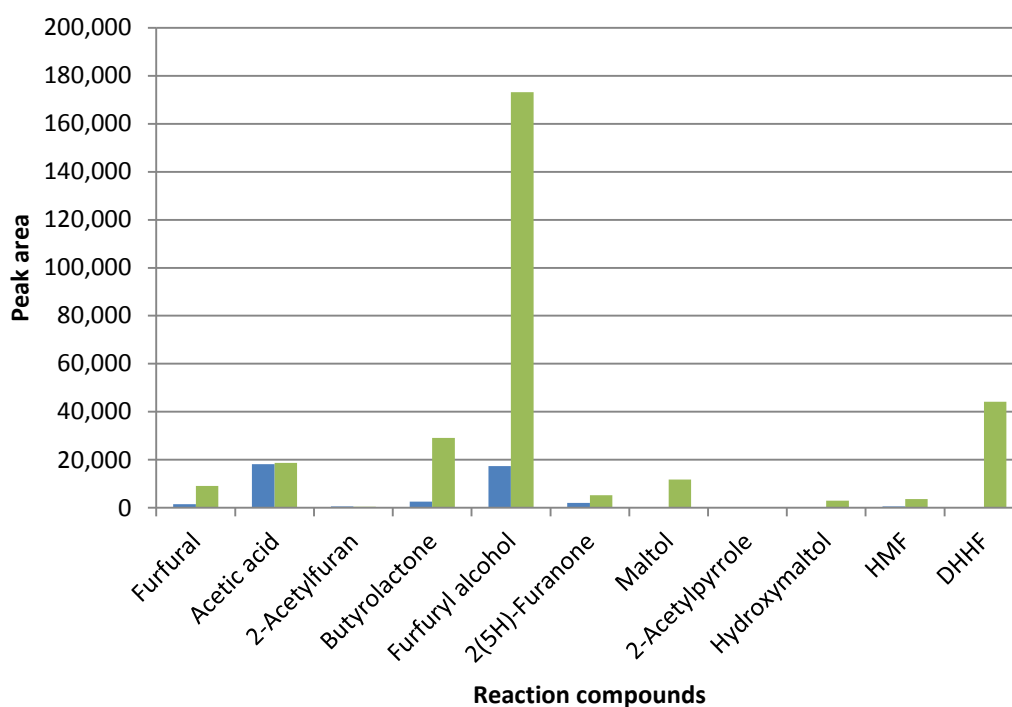


Figure 4.18 Impact of phosphate buffer addition of the caramelisation reaction. Caramelisation reaction samples containing 0.2 M phosphate buffer (pH 7) shown by the green bars and samples without phosphate buffer shown by the blue bars. This experiment was not replicated.

When phosphate buffer was added to the caramelisation reaction an increase in 2,3-enolisation compounds, maltol, hydroxymaltol and DHMF was detected. The formation of these compounds highlighted the impact that the phosphate groups or buffering effect could be having on the reaction as these compounds had not previously (figure 4.5) been seen under these cooking conditions.

The addition of salts such as sodium chloride did not appear to have the same effect on either reaction. The addition of 5% sodium chloride to both the Maillard reaction (figure 4.19) and the caramelisation reaction (figure 4.20) did not result in a change in relative amounts of volatile compounds that were formed as large as that seen when the phosphates were added to the system.

The addition of sodium chloride to the Maillard reaction lead to lower formation of volatile compounds with the exception of furfuryl alcohol and 2(5H)-furanone. A similar general decrease in compound formation was seen in the caramelisation reaction; however, increases in furfural and 2-acetylfuran were detected.

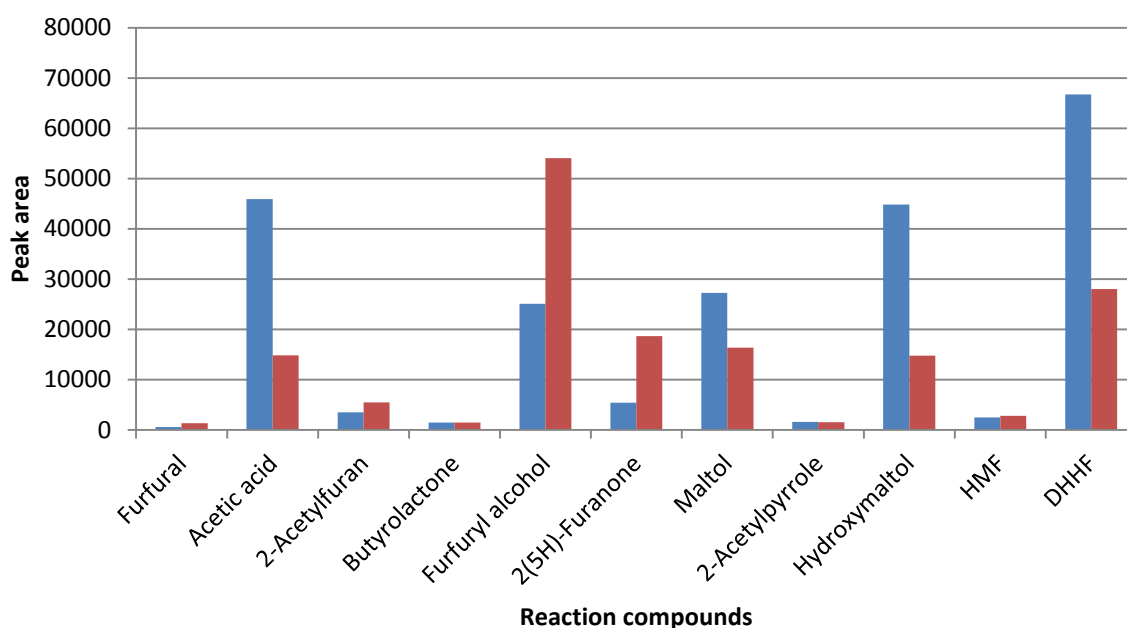


Figure 4.19 Influence of sodium chloride (5%) on the Maillard reaction. Maillard reaction samples containing sodium chloride are shown by the red bars, samples without sodium chloride are shown by the blue bars.

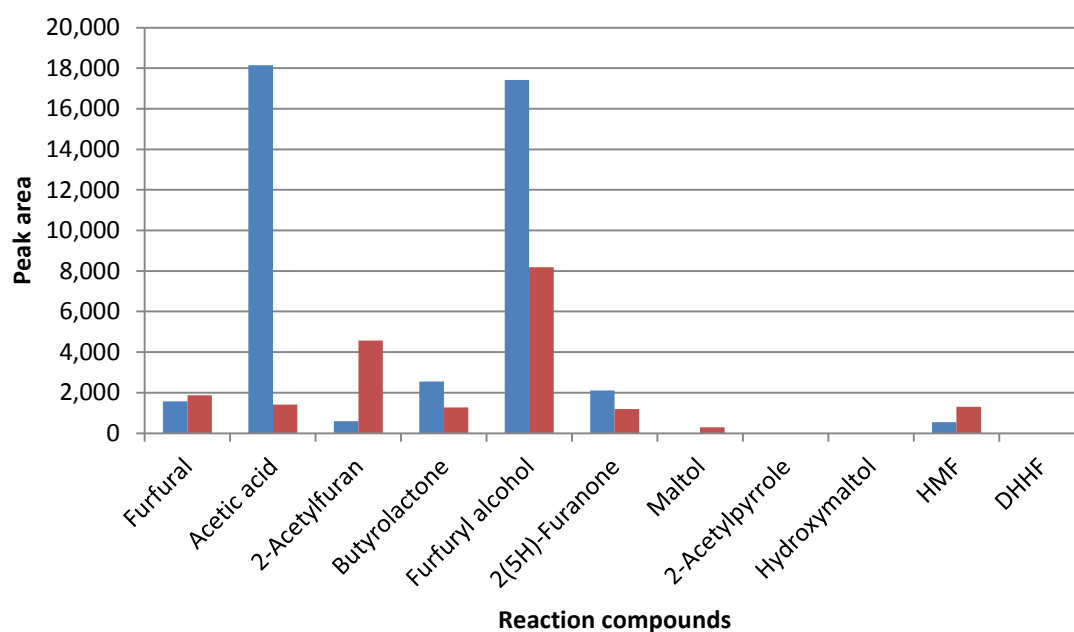


Figure 4.20 Influence of sodium chloride on the caramelisation reaction. Caramelisation reaction samples containing sodium chloride are shown by the red bars, samples without sodium chloride are shown by the blue bars.

The increase of the amount of some compounds formed when phosphates are present and the general decrease in compound formation when sodium chloride was added to the system demonstrates the different influences that the salt components of cream can have on the system. Additionally, salts can influence the structure of the matrix as well as the reactions (38). Therefore for the purposes of keeping the reactions as simple as possible, the salts were not added to the model reaction when changing the structural matrix.

4.7 Conclusion

The reactions occurring in cream while it is being cooked were modelled based on the caramelisation reaction and the Maillard reaction. These model reaction systems were simplified to lactose and lysine in an effort to remove the majority of further confounding chemical factors. Based on experimental evidence, these reaction systems behaved in a similar way to cream when different parameters such as time, temperature and pH were changed. Differences that were seen in the results can be explained, in part, by the presence of buffers and salts in cream, the differences may be able to be further explained by the presence of lipids and the emulsion structure of cream. The next step was to establish the model structural matrix, using a step by step build so that each part of the matrix could be studied and its role determined in a systematic fashion.

As described in chapters 5 and 6, building the aqueous matrix into a cream-like matrix involved adding several structural components: an oil phase following by an emulsion structure containing emulsifiers. The contribution of each component and each of the various parameters associated with these components was explored using the model reactions developed in this chapter. The influence of the addition of fat on the reaction outcome can be found in chapter 5, followed by a study into the influence of emulsion structure in chapter 6.

4.8 References

1. Ajandouz, E. H.; Tchiakpe, L. S.; Dalle Ore, F.; Benajiba, A.; Puigserver, A., Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science* **2001**, *66*, 926-931.
2. Ajandouz, E. H.; Desseaux, V.; Tazi, S.; Puigserver, A., Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry* **2008**, *107*, 1244-1252.
3. van Boekel, M. A. J. S., Effect of heating on Maillard reactions in milk. *Food Chemistry* **1998**, *62*, 403-414.
4. Rizzi, G. P., Effects of cationic species on visual color formation in model Maillard reactions of pentose sugars and amino acids. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 7160-7164.
5. Philippe, E.; Seuvre, A. M.; Colas, B.; Langendorff, V.; Schippa, C.; Voilley, A., Behavior of flavor compounds in model food systems: a thermodynamic study. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 1393-1398.
6. Brands, C. M. J.; van Boekel, M., Reactions of monosaccharides during heating of sugar-casein systems: Building of a reaction network model. *Journal of Agricultural and Food Chemistry* **2001**, *49*, 4667-4675.
7. Nursten, H. E., *The Maillard reaction: chemistry, biochemistry and implications*. RSC Publishing: Cambridge, UK, 2005.
8. Ledl, F.; Schleicher, E., New aspects of the Maillard reaction in foods and in the human body. *Angewandte Chemie - International Edition* **1990**, *29*, 565-594.
9. Gerrard, J. A., New aspects of an AGEing Chemistry - Recent developments concerning the Maillard reaction. *Australian Journal of Chemistry* **2002**, *55*, 299-310.
10. van Boekel, M. A. J. S., Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances* **2006**, *24*, 230-233.
11. Berg, H. E.; Vanboekel, M., Degradation of lactose during heating of milk. 1. Reaction pathways. *Netherlands Milk and Dairy Journal* **1994**, *48*, 157-175.
12. Aider, M.; de Halleux, D., Isomerization of lactose and lactulose production: review. *Trends in Food Science and Technology* **2007**, *18*, 356-364.
13. van Boekel, M. A. J. S.; Brands, C. M. J., Heating of sugar-casein solutions: Isomerization and Maillard reactions. In *The Maillard reaction in foods and medicine*, O'Brien, J.; Nursten, H. E.; Crabbe, M. J.; Ames, J. M., Eds. Woodhead publishing: Cambridge, UK, 1998; pp 154-159.
14. van Boekel, M. A. J. S., Kinetic modelling of sugar reactions in heated milk-like systems. *Netherlands Milk and Dairy Journal* **1996**, *50*, 245-266.
15. Wang, Y.; Juliani, H. R.; Simon, J. E.; Ho, C. T., Amino acid-dependent formation pathways of 2-acetylfuran and 2,5-dimethyl-4-hydroxy-3(2H)-furanone in the Maillard reaction. *Food Chemistry* **2009**, *115*, 233-237.

16. Wang, Y.; Ho, C. T., Comparison of 2-acetylfuran formation between ribose and glucose in the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 11997-12001.
17. Shaw, P. E.; Tatum, J. H.; Berry, R. E., 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one, A degradation product of a hexose. *Carbohydrate Research* **1971**, *16*, 207-213.
18. Kim, M. O.; Baltes, W., On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one in the Maillard reaction. *Journal of Agricultural and Food Chemistry* **1996**, *44*, 282-289.
19. Parker, J. K., The kinetics of thermal generation of flavour. *Journal of the Science of Food and Agriculture* **2013**, *93*, 197-208.
20. Shimamura, T.; Kurogi, Y.; Katsuno, S.; Kashiwagi, T.; Ukeda, H., Demonstration of the presence of aminoreductone formed during the Maillard reaction in milk. *Food Chemistry* **2011**, *129*, 1088-1092.
21. Trang, V. T.; Shimamura, T.; Kashiwagi, T.; Ukeda, H.; Katsuno, S., Elucidation of mechanism of aminoreductone formation in the Maillard reaction of lactose. *International Journal of Dairy Technology* **2011**, *64*, 188-196.
22. Pischetsrieder, M.; Schoetter, C.; Severin, T., Formation of an aminoreductone during the Maillard reaction of lactose with N-alpha-acetyllysine or proteins. *Journal of Agricultural and Food Chemistry* **1998**, *46*, 928-931.
23. TheGoodScentsCompany, Flavours and fragrances. In <http://www.thegoodscentscompany.com/>, 2012.
24. Wong, K. H.; Aziz, S. A.; Mohamed, S., Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. *International Journal of Food Science and Technology* **2008**, *43*, 1512-1519.
25. Davidek, T.; Devaud, S.; Robert, F.; Blank, I., Sugar fragmentation in the Maillard reaction cascade: Isotope labeling studies on the formation of acetic acid by a hydrolytic beta-dicarbonyl cleavage mechanism. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 6667-6676.
26. Keogh, M. K., Anhydrous milk fat. 5. Deep fat frying. *Irish Journal of Food Science and Technology* **1989**, *13*, 129-140.
27. Shrestha, A. K.; Howes, T.; Adhikari, B. P.; Bhandari, B. R., Spray drying of skim milk mixed with milk permeate: Effect on drying behavior, physicochemical properties, and storage stability of powder. *Drying Technology* **2008**, *26*, 239-247.
28. Martins, S.; Van Boekel, M., Kinetics of the glucose/glycine Maillard reaction pathways: influences of pH and reactant initial concentrations. *Food Chemistry* **2005**, *92*, 437-448.
29. Yu, A. N.; Zhang, A. D., The effect of pH on the formation of aroma compounds produced by heating a model system containing L-ascorbic acid with L-threonine/L-serine. *Food Chemistry* **2010**, *119*, 214-219.
30. Pauletti, M. S.; Matta, E. J.; Rozycki, S., Kinetics of heat-induced browning in concentrated milk with sucrose as affected by pH and temperature. *Food Science and Technology International* **1999**, *5*, 407-413.

31. Salaun, F.; Mietton, B.; Gaucheron, F., Buffering capacity of dairy products. *International Dairy Journal* **2005**, *15*, 95-109.
32. Novotny, O.; Cejpek, K.; Velisek, J., Formation of carboxylic acids during degradation of monosaccharides. *Czech Journal of Food Sciences* **2008**, *26*, 117-131.
33. Smuda, M.; Voigt, M.; Glomb, M. A., Degradation of 1-deoxy-D-erythro-hexo-2,3-diulose in the presence of lysine leads to formation of carboxylic acid amides. *Journal of Agricultural and Food Chemistry* **2010**, *58*, 6458-6464.
34. Hajek, J.; Murzin, D. Y.; Salmi, T.; Mikkola, J. P., Interconversion of lactose to lactulose in alkaline environment: comparison of different catalysis concepts. *Topics in Catalysis* **2013**, *56*, 839-845.
35. Ganzle, M. G.; Haase, G.; Jelen, P., Lactose: Crystallization, hydrolysis and value-added derivatives. *International Dairy Journal* **2008**, *18*, 685-694.
36. van Boekel, M. A. J. S., Kinetic aspects of the Maillard reaction: a critical review. *Nahrung - Food* **2001**, *45*, 150-159.
37. Bell, L. N., Maillard reaction as influenced by buffer type and concentration. *Food Chemistry* **1997**, *59*, 143-147.
38. Zhang, J. C.; Li, L.; Wang, J.; Sun, H. G.; Xu, J.; Sun, D. J., Double inversion of emulsions induced by salt concentration. *Langmuir* **2012**, *28*, 6769-6775.

Chapter 5 The role of fat in the Maillard and caramelisation reactions

5.1 Introduction

This chapter takes the model reaction systems developed in chapter 4 and introduces a fat component as part of the colloidal structure to probe the role of fat when the reaction systems are cooked at high temperatures. In the context of this study the term fat covers both liquid oils and solid lipids of plant and animal origins. Fat present in a food system can have various roles as part of the material functionality, flavour perception, texture and the health attributes of that system (1).

The properties and functionality of fats are determined by their fatty acid components, which have different levels of saturation and different chain lengths (1). The length of the chains and degree of saturation affect the melting point and the crystal structure of the fat at different temperatures. Addition of fat to a food matrix or reaction matrix has the potential to change the properties of the overall system and, therefore, the course of the reaction or behaviour of a reaction in the selected matrix. The properties of a system that change upon the addition of fat may include the polarity, the specific heat capacity and mass transfer parameters. It has also been suggested that particular free fatty acids can have an influence on compound formation (2, 3).

Fat has a low polarity and is hydrophobic; therefore, the addition of fat to an aqueous matrix will generally provide a second phase. This second phase has properties substantially different from those of the initial water phase, which leads to the partitioning and mass transfer of reactants, reaction intermediates and final volatile compounds across the two phases (4, 5). The partition coefficient of each compound will determine which of the two phases the compounds are in. Milk fat and plant oils have different chemical compositions and physical properties and so differences in partitioning are very likely. The partitioning of reaction products into the fat phase may move the position of equilibrium in the aqueous phase and so lead to product formation (5-7).

Canola oil and anhydrous milk fat (AMF) were the fats chosen for addition to the model reaction system as set out in section 8.6. Anhydrous milk fat, derived from dairy products, contains a large percentage of shorter saturated fatty acid chains, and usually needs to be heated above 40 °C for all the fat to be melted (Fonterra product bulletin, appendix B). Canola oil has a higher percentage of longer unsaturated fatty acid chains and is a liquid at room temperature (8). These two fats also have different densities, specific heat capacities and thermal conductivity properties (8). The addition of fat to the aqueous reaction created a reaction environment that contained two phases each with different physical properties, which will be referred to as the two phase system. The use

of two different fats allowed the impact of the particular properties of that fat on the reactions to be investigated as well as the general impact of fat addition.

The effect of changes in heat transfer throughout a reaction system along with moisture evaporation need to be considered as the addition of fat (and the specific properties of the fat) will have an influence over these parameters, which in turn can influence the Maillard and caramelisation reactions (9). It has already been shown that the formation of volatile compounds in ghee (section 3.4) and the model systems (section 4.4) changes with cooking temperature. However, the internal temperature of the system is not always the same as the set cooking temperature (10). The internal temperature of the sample relies on the heat transfer of the system as determined by the thermal properties of the reaction medium (11).

A considerable amount of research has been undertaken on the participation of lipids and the carbonyl groups of oxidised lipids in the Maillard reaction (12-15). The work in this chapter looks at the influence of fat on the physical parameters of the reaction system that influence the Maillard and caramelisation reactions to generate flavours other than those arising from methyl ketones and aldehydes which are generated from fats (section 1.3.5).

5.2 The influence of fat on the Maillard reaction

The PCA plot (figure 5.1) comparing samples (chapter 8, section 8.6) cooked in a 100% aqueous reaction medium and those cooked in a 60% aqueous medium with added fat indicates a clear difference in the formation of volatile compounds. The addition of the fat to the matrix generated a significant difference (p value <0.05, as determined by ANOVA (appendix C, table C1)) and figure 5.2 demonstrates that the difference generated was an overall increase in volatile compound intensity within the cooked sample. A significant increase was seen in most of the compounds measured in the Maillard model reaction system, the exceptions being acetic acid, hydroxymaltol and DHMF.

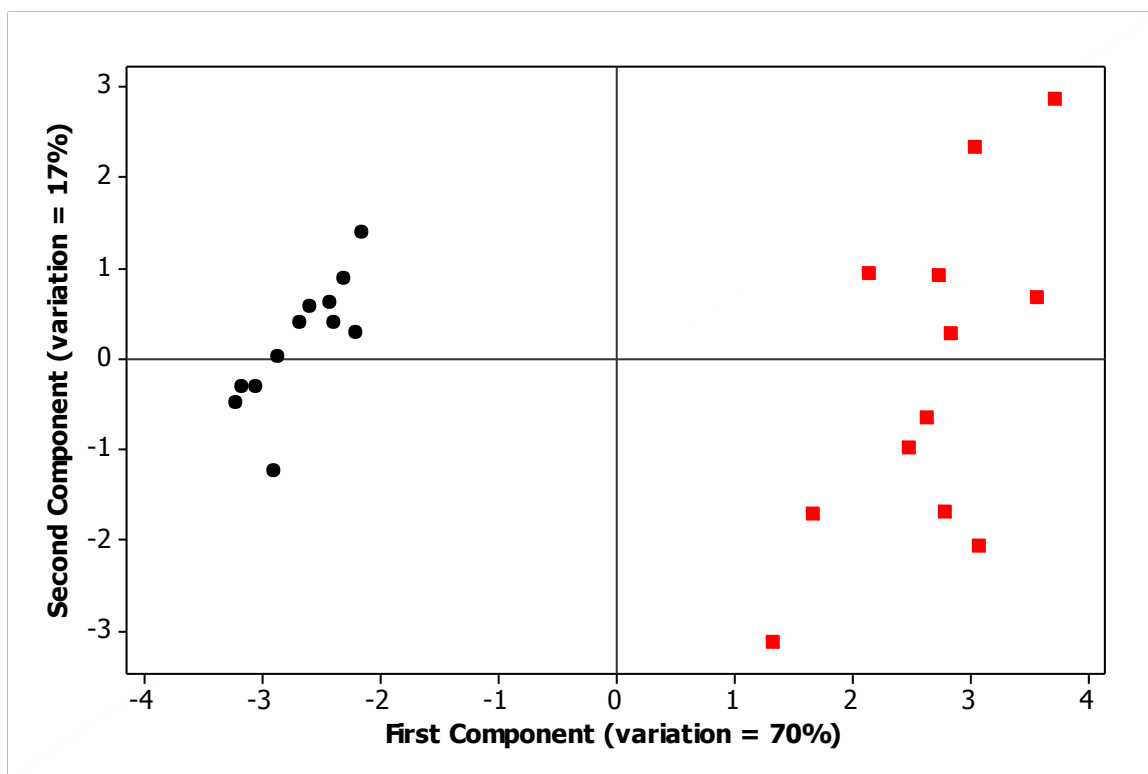


Figure 5.1 PCA of the influence of fat on the Maillard reaction. Samples containing 40% fat and 60% aqueous phase are shown by red squares and samples with 100% aqueous phase are black circles.

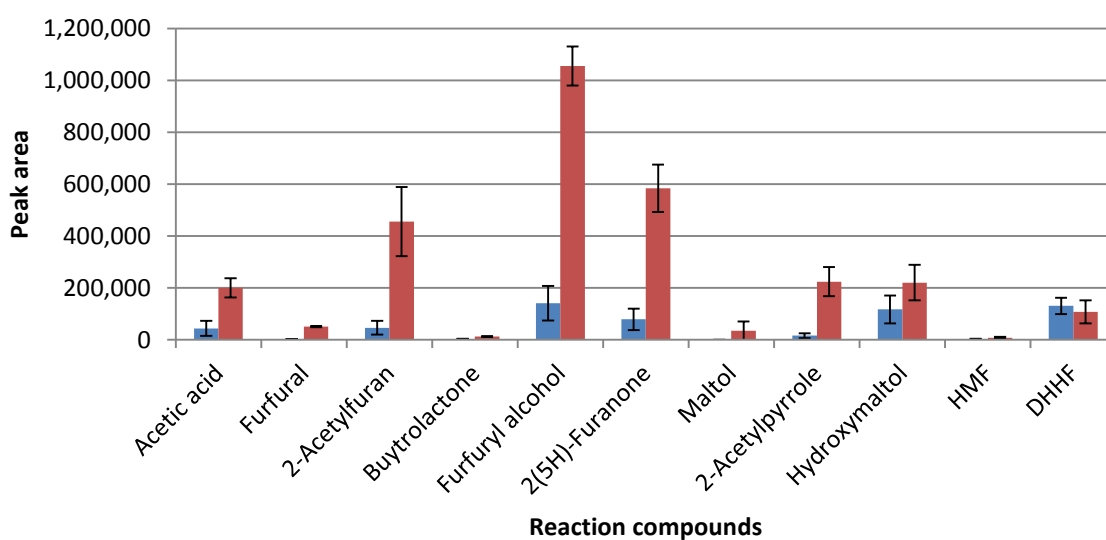


Figure 5.2 Influence of fat (samples containing canola oil shown by red bars, samples containing no canola oil shown by blue bars) on the formation of volatile compounds by the Maillard reaction (Error is based on standard deviation of six samples).

When the samples containing fat were separated into samples containing canola oil and samples containing anhydrous milk fat (AMF) in the principal components plot (figure 5.3) a pattern emerged that suggested there were some variations in the amounts of volatile compounds generated as the type of fat was changed. The changes that occurred when a different fat was used were smaller than those achieved when fat was initially added to the aqueous system. The significance of the differences in the amounts of volatile compound formed when using AMF and canola oil was determined using ANOVA testing to calculate the p value (appendix C, table C2). The compounds whose formation was significantly altered when the oil was changed from canola oil to AMF were hydroxymaltol, 2-acetylpyrrole, furfuryl alcohol, butyrolactone and furfural.

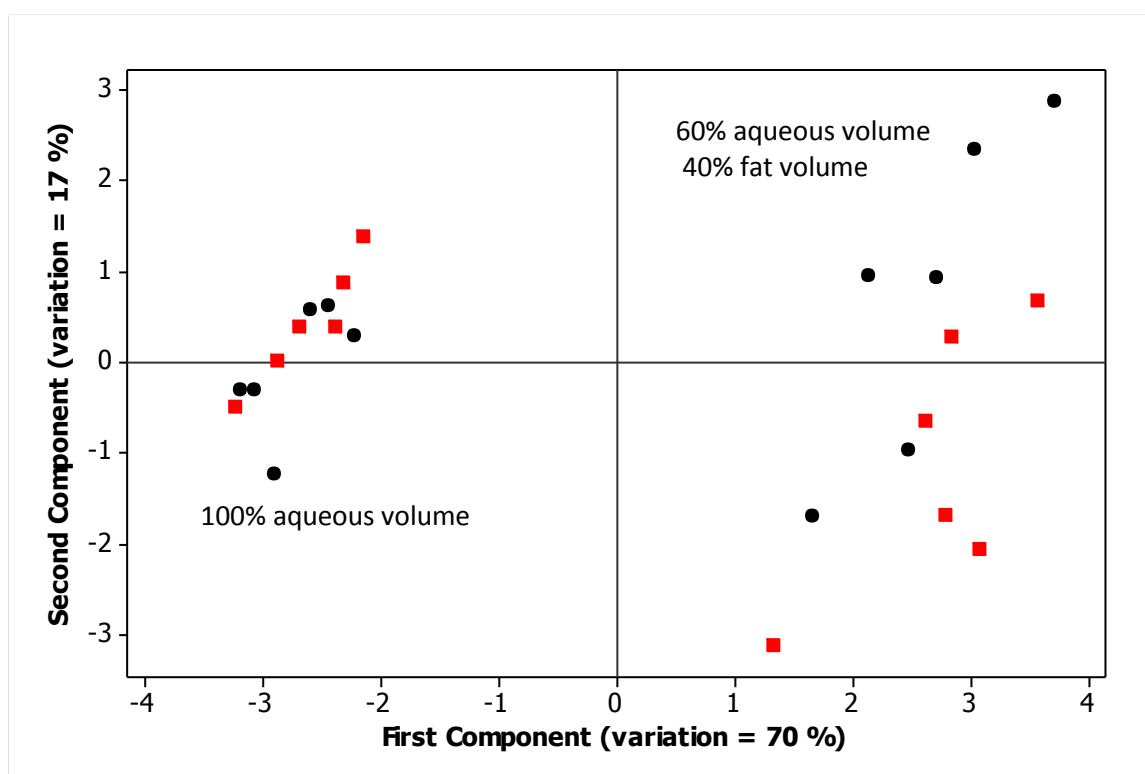


Figure 5.3 PCA plot of the Maillard reaction model system with two different fats added. Samples are identified by the fat used for cooking and analysis: AMF (black circles) and canola oil (red squares). Samples labelled 100% aqueous volume were cooked without fat.

It was established using ANOVA testing and p values (appendix C, table C2) that several compounds were formed in significantly different yields when the oil was changed (figure 5.4) namely: hydroxymaltol, 2-acetylpyrrole, furfuryl alcohol, butyrolactone and furfural. Of these compounds, the levels of some (furfural, furfuryl alcohol and butyrolactone) were increased when canola oil was

used instead of AMF, whilst the levels of other compounds (hydroxymaltol and 2-acetylpyrrole) decreased with the use of canola oil.

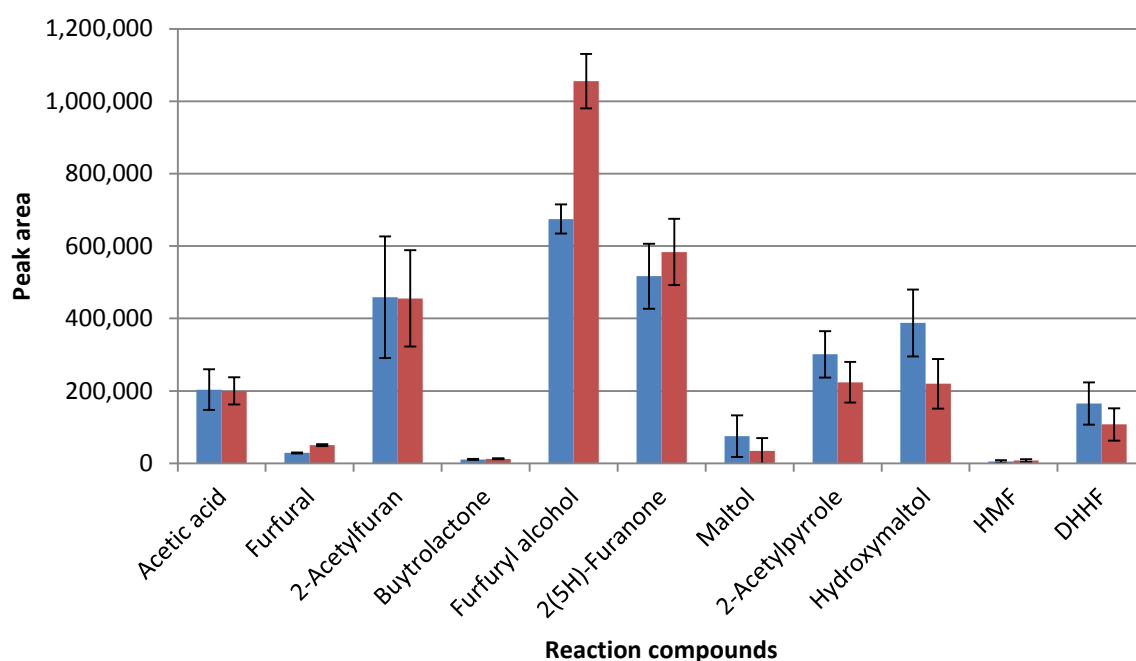


Figure 5.4 Influence of fat type on the Maillard reaction. Error bars are the standard deviation of replicate samples for this experiment. Maltol values are underestimated due to poor peak shape. Blue bars represent samples containing AMF as the fat phase, and samples containing canola oil are shown in red. The error bars represent the standard deviation of six replicate samples.

5.3 The influence of fat on the caramelisation reaction

The caramelisation reaction behaved differently to the Maillard reaction when fat was added to the system (figure 5.5). The difference between the samples with fat and without fat was not as large when plotted on a PCA plot and compared to the differences seen in the Maillard reaction (figure 5.1).

When fat (canola) was added to the caramelisation model (figure 5.6) several compounds exhibited a significant change including DHMF, furfuryl alcohol, butyrolactone, 2-acetylfuran, furfural and acetic acid (appendix C, table C3). Of these compounds, furfural and 2-acetylfuran increased in yield when the fat was added, whilst DHMF, furfuryl alcohol, butyrolactone and acetic acid decreased when fat was added to the system.

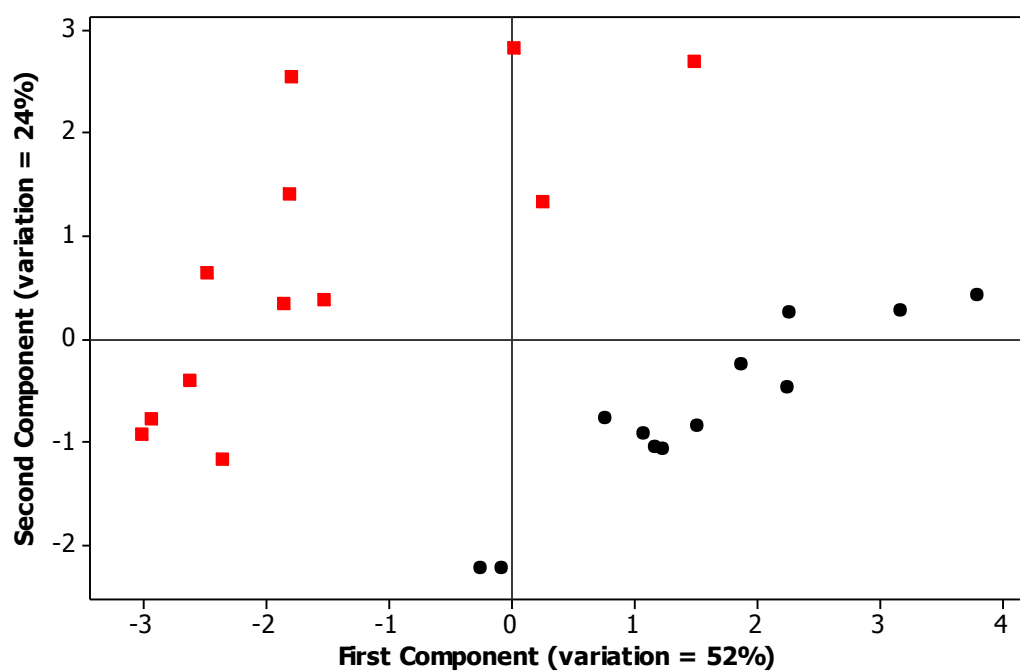


Figure 5.5 PCA plot of the differences between caramelisation reaction samples containing fat (red squares) and samples without fat (black circles).

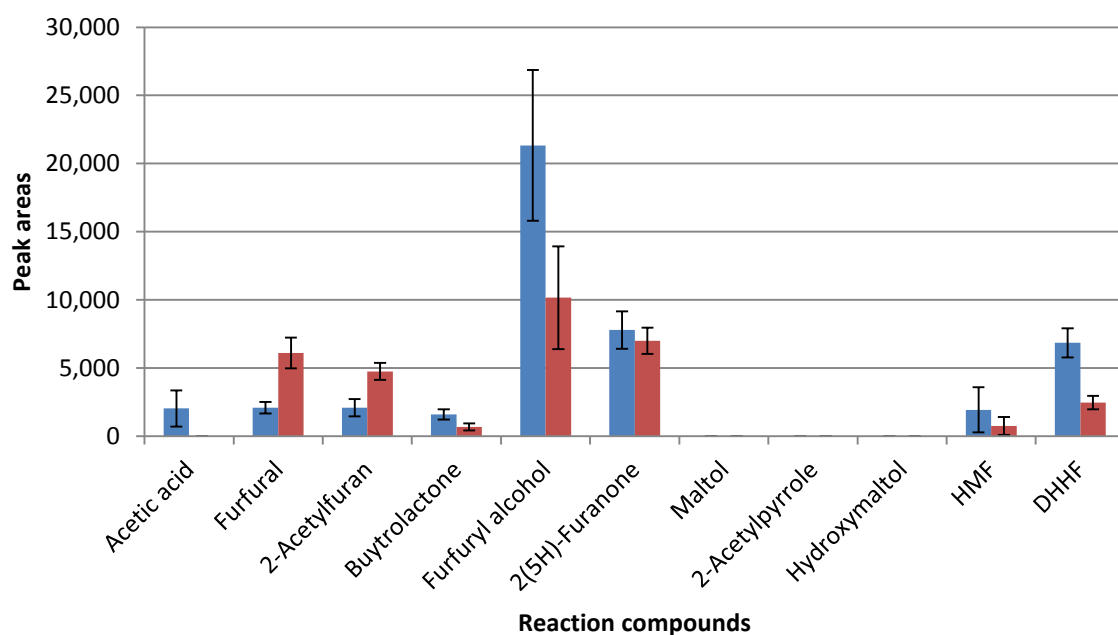


Figure 5.6 The addition of fat (canola) to the caramelisation reaction matrix. Samples with 100% aqueous phase are shown in blue while samples containing 60% aqueous phase plus canola oil are shown in red. Error bars are based on the standard deviation of six samples within the experiment.

When considering the impact of fat on the caramelisation reaction (figure 5.6) the addition of fat appears to have significant impact on some of the compounds, with acetic acid, furfural, 2-acetylfuran, butyrolactone, furfuryl alcohol and DHHF having a calculated p value <0.05 when fat was added to the system (appendix C, table C3). When a closer look is taken at the PCA plot (figure 5.7) to determine the impact of the different types of fat (AMF versus canola) it appears that there is a shift in the overall compound profile. However, it is only a small shift when compared with the overall shift in the profile generated by the addition of fat to the aqueous system.

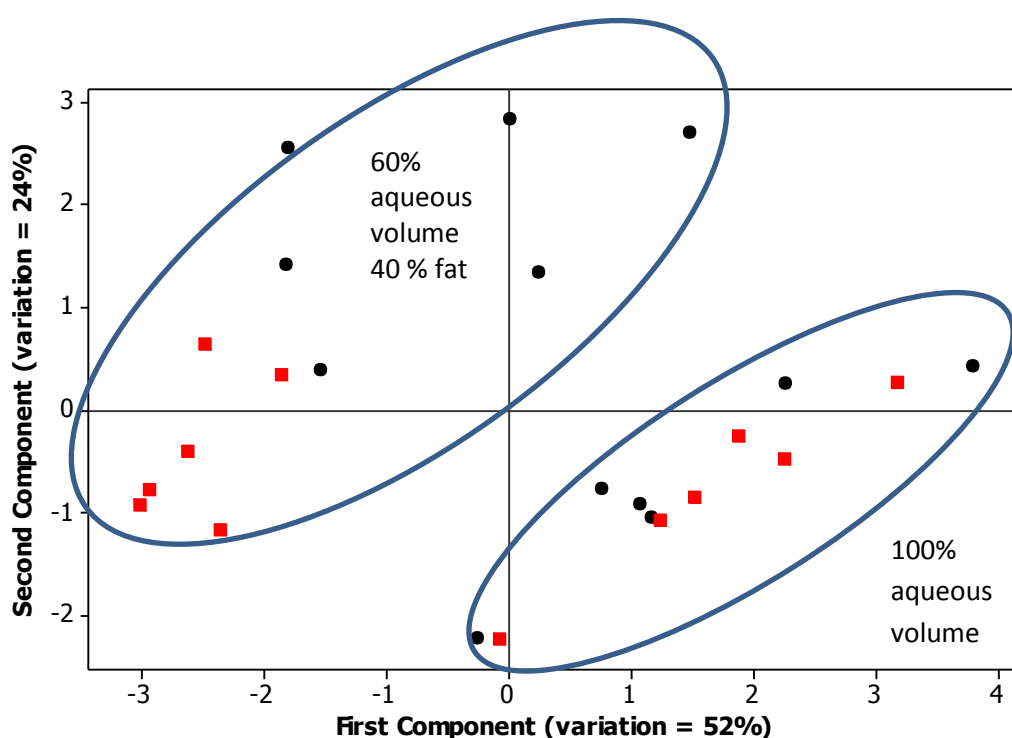


Figure 5.7 PCA plot of the influence the type of fat has on the caramelisation reaction. AMF is represented by the black circles, canola oil samples are represented by red squares.

The change in compound profile identified in the PCA plot (figure 5.7) was due to the significant shifts in the levels of acetic acid, 2(5H)-furanone, furfuryl alcohol and buytrolactone formed (appendix C, table C4). The amounts of these compounds all decreased when canola oil was used instead of AMF (figure 5.8). The changes in the caramelisation reaction with the addition of fat and by changing the type of fat were small compared with the changes occurring in the Maillard reaction (figure 5.9).

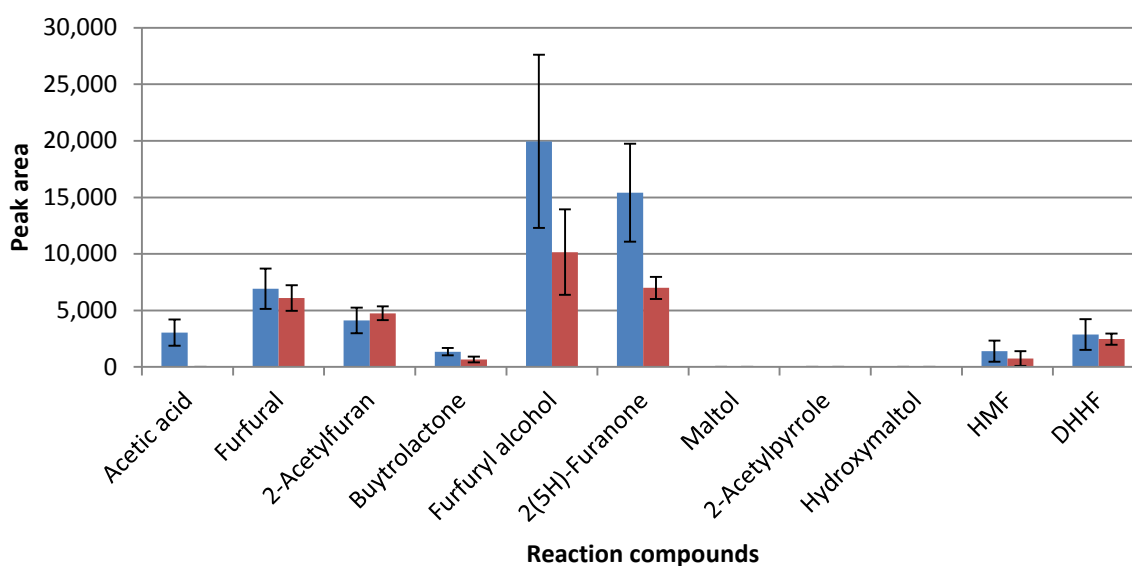


Figure 5.8 Changes occurring in the formation of the volatile compounds when the fat phase of the caramelisation reaction was changed from canola oil (red bars) to AMF (blue bars). The error bars represent the standard deviation of six replicate samples.

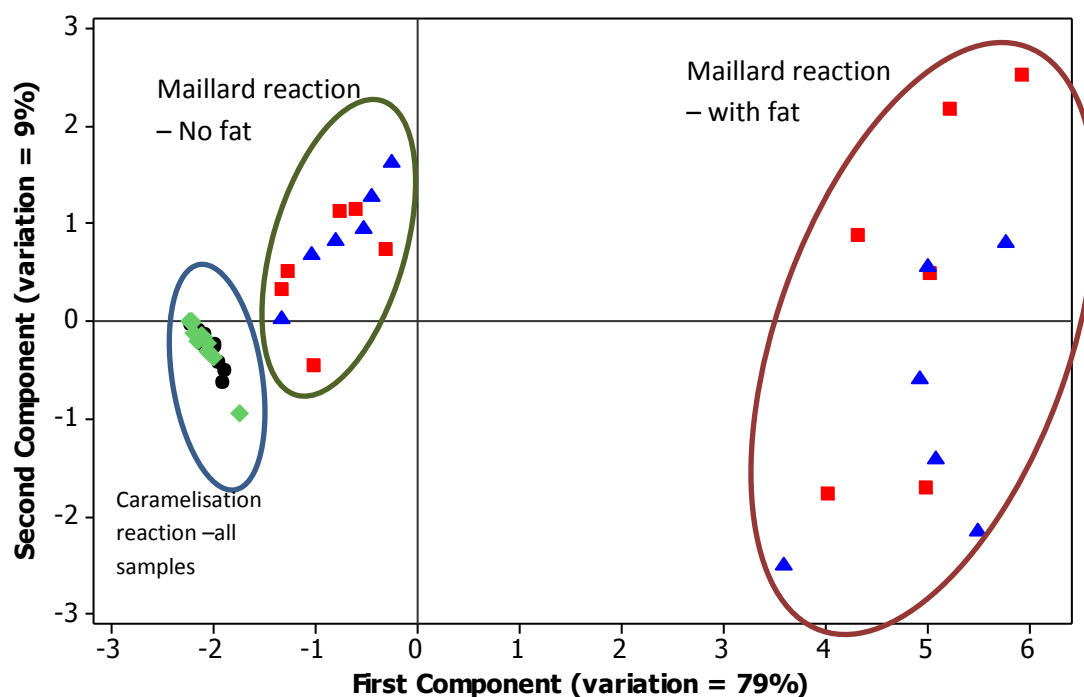


Figure 5.9 PCA plot of the variation in compound profiles achieved using caramelisation reaction versus Maillard reaction, aqueous versus two phase matrix and different fats. The caramelisation reaction is shown in green diamonds (canola oil) and black circles (AMF) with the Maillard reaction represented by red squares (AMF) and blue triangles (canola oil).

5.4 Aqueous concentration effect

The model reactants were dissolved in the aqueous portion of the reaction environment. The total reactant concentration was kept the same for both the aqueous and two phase samples. This was achieved by increasing the reactant concentration in the aqueous phase of the two phase samples due to the lack of reactant solubility in the fat. Therefore, each sample contains the same quantity of reactants; however, they are dissolved in a different volume. The aqueous matrix samples have an aqueous volume of 100% and the two phase matrix samples have an aqueous volume of 60%.

Control testing (chapter 8, section 8.6.1) on the different aqueous concentrations (both contain 0.272 mmol lysine and/or 0.174 mmol lactose per 2 ml or 1.2 ml sample) showed that the aqueous concentration had no significant impact on the overall results of the Maillard (figure 5.10) (appendix C, table C5) and caramelisation reactions (figure 5.11) (appendix C, table C6). Any of the monitored compounds that were influenced by the concentration of the aqueous phase were expected to increase in yield at higher aqueous concentrations. However, there were no large increases in the formation of volatile compounds across either the Maillard (figure 5.10) or caramelisation (figure 5.11) reactions indicating that the aqueous concentration was not an influencing factor for the model reaction system under these conditions.

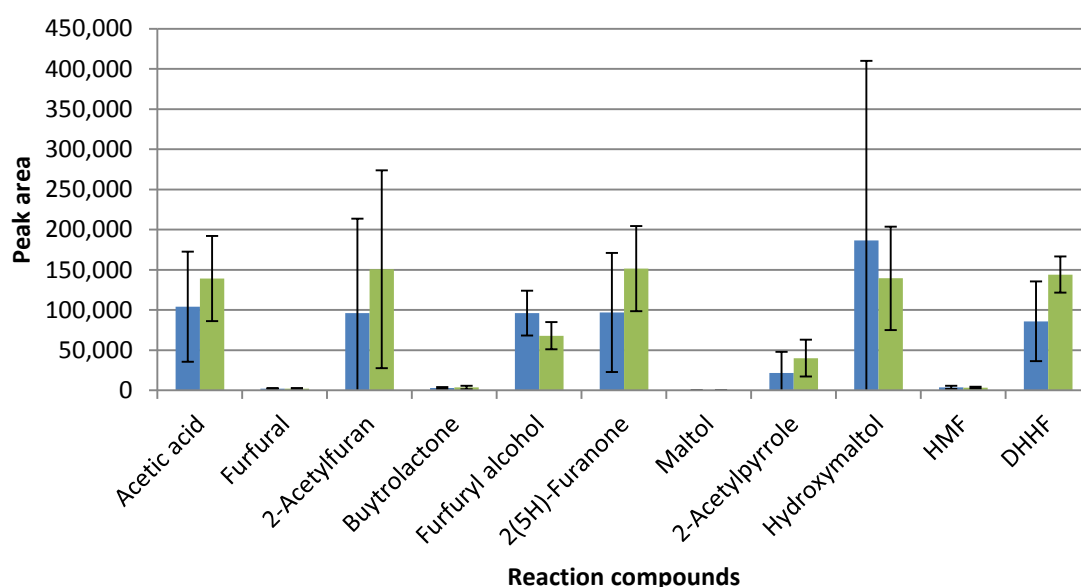


Figure 5.10 Aqueous concentration effects on the Maillard reaction. Samples containing 0.174 mmol lactose and 0.272 mmol lysine in 2 ml shown as blue bars and samples containing the same quantities in 60% volume (1.2 ml) are shown as green bars. Error bars represent the standard deviation of six replicate samples.

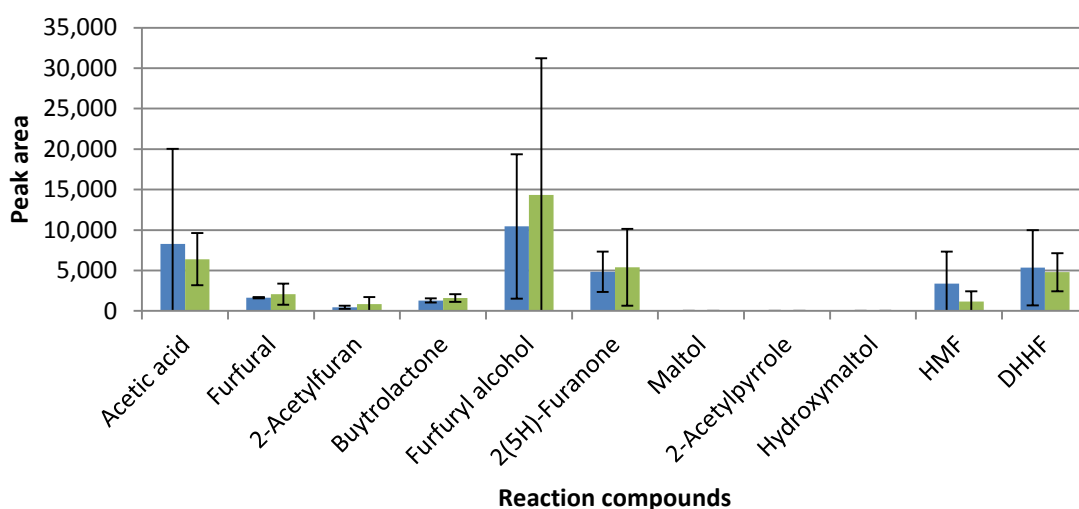


Figure 5.11 Aqueous concentration effects on the caramelisation reaction. Samples containing 0.174 mmol lactose in 2 ml are shown as blue bars and samples containing the same quantities in 60% volume (1.2 ml) are shown as green bars. The error bars represent the standard deviation of six replicate samples.

5.5 Reaction temperature and evaporation

The increase in formation of volatile compounds across the cross section of selected compounds suggests that the reaction rates of the initial stages of the Maillard reaction increased with the addition of the fat. The addition of fat provided the reaction matrix with a solvent that unlike water does not evaporate as the temperature approaches 135 °C. When the reactants were heated in a 100% aqueous system the water evaporated as the temperature reached 100 °C to leave only solid reactants behind. This complete evaporation of the water potentially creates a situation where the reactants may have limited mobility and are being dry roasted together (16, 17). When the two phase system was heated the water evaporated but the fat remained. The fat provided a solvent environment where the reactants and intermediate compounds have a degree of mobility that may lead to a higher rate of particular reactions and correspondingly the higher product yields that were seen in the results. Figure 5.12 provides an indication of the rate of evaporation overlaid with the temperature profile of the system. (See chapter 8, section 8.6.2 for experimental details.) This temperature/time profile was the same for both the caramelisation and Maillard reaction samples.

The Maillard reaction is directly influenced by cooking temperature (18-20) and although the cooking temperature was maintained at 135 °C the internal temperature of the sample being cooked was not necessarily the same as the applied external cooking temperature (10). The heat transfer

properties of the matrix determines the temperature inside the sample (9, 21) and it is expected that heat transfer would become more important as the sample size increases relative to the heating surface. Therefore for the small samples that were utilised for these experiments the temperature gradient across the sample it is expected that would be small. The influence of temperature on the aqueous model reaction system is shown in figure 4.6, where the final compound yields were increased with increasing temperature. Figure 5.12 demonstrates how the internal temperature changed in the aqueous samples when compared to the two phase samples as the cooking time progressed and the water and other volatiles evaporated from the reaction matrix. After an initial rise in temperature to $\sim 100^\circ\text{C}$, the temperature plateaus until the water has evaporated from the system. The water from the two phase sample evaporated several minutes earlier than the 100% aqueous sample and therefore the temperature of the sample increased above 100°C after a shorted time period. This prolonged increase in temperature leads to an increase in the rate of the Maillard reaction.

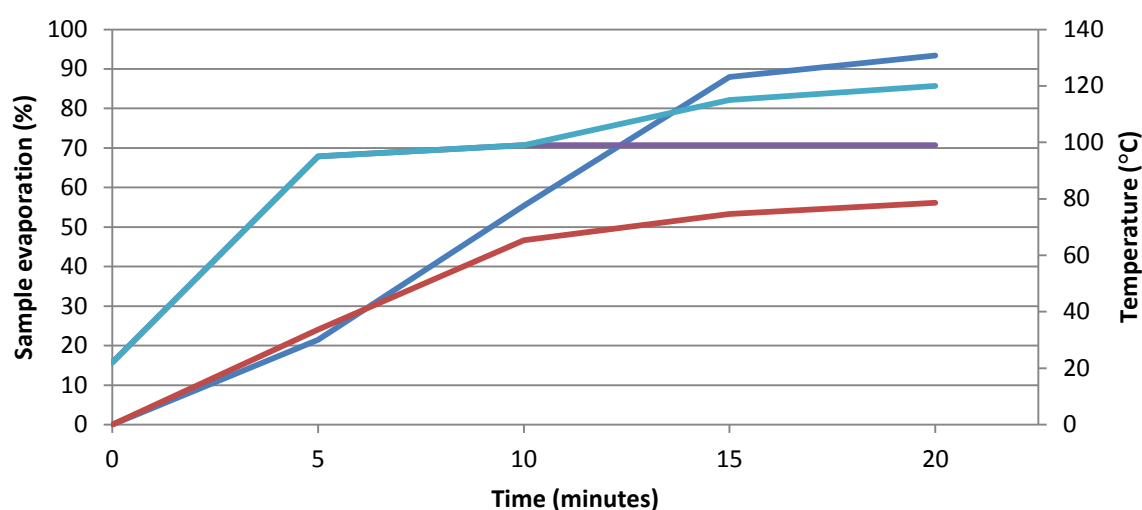


Figure 5.12 Evaporation and temperature profile of the samples in different matrices (cooked at 135°C for 20 min with stirring). The internal temperature of aqueous samples is shown in purple with temperature of the two phase samples in pale blue. The corresponding evaporation of the aqueous samples is shown in dark blue with that of the two phase samples represented in red. The two phase matrix contains canola oil.

The time of the increase in temperature of the two phase system (figure 5.12) corresponds to the time at which product formation in the Maillard reaction increased (figure 5.13a). Comparatively the

formation of volatile compounds in the aqueous system was much slower and occurred several minutes later in the cooking process (figure 5.13b).

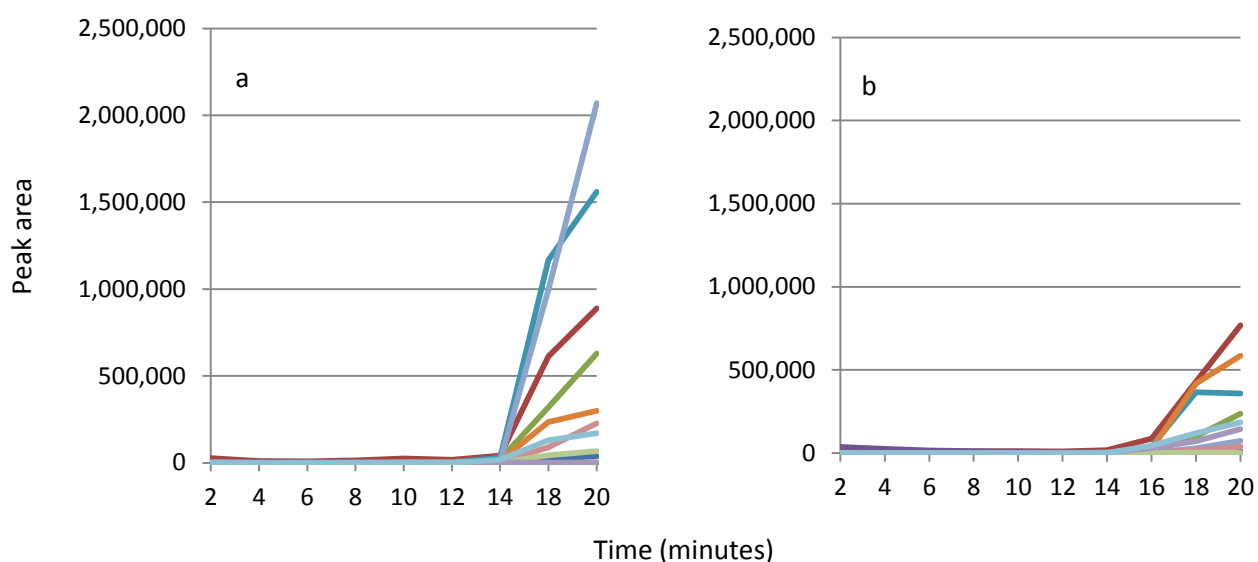
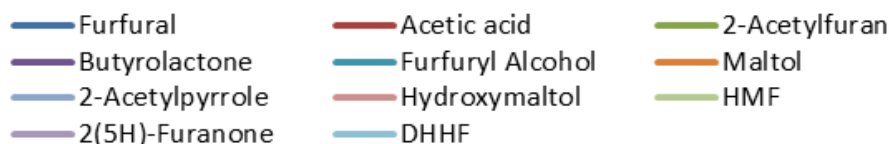


Figure 5.13 (a) Maillard reaction time series for the two phase reaction (60% aqueous, 40% fat).
(b) Maillard reaction time series plot for 100% aqueous reaction.



5.6 Participation of fat in the Maillard reaction

Although the fat was added to the reaction matrix as a structural component and changed the physical properties of that matrix, there was also the possibility that there were reactions occurring between the dicarbonyl groups of lipid oxidation products and the amines (12-14, 22). The Maillard model system has an excess of amino groups that could react with the dicarbonyl groups. Such reactions could offer a possible explanation as to why the changes observed in the caramelisation reaction were smaller when fat was added, as there are no amino groups for the lipid oxidation products to interact with.

The results generated when the type of fat used in the Maillard reaction system was changed from AMF to canola oil indicated a change in volatile compound formation and for some compounds this was a significant change. Canola oil consists of a large percentage of unsaturated C18 fatty acids (8)

that have been studied for their impact on flavour formation in meat-like model systems (2) in which it was found that different degrees of unsaturation could inhibit or promote the Maillard reaction.

Therefore a set of reaction samples were set up containing only lysine in the two phase system (canola oil) to investigate the potential of product formation via reaction with any lipid oxidation products under the standard reaction conditions. The experiment indicated that none of the Maillard reaction products that were monitored were formed at detectable levels (data not shown).

5.7 The partitioning effect of the two phase reaction matrix

The changes seen in the reaction compound profiles when fat was added to the Maillard reaction, and to a lesser degree to the caramelisation reaction, can be attributed to the changes in the physical properties of the reaction matrix. The properties of the reaction matrix may have the capability to alter the final compound profile by changing the reactant and intermediate compound partitioning within the reaction matrix and therefore provide an explanation as to why the change in volatile compound yield was seen.

The introduction of the hydrophobic fat phase leads to a potential partitioning effect between the fat phase and the water phase. The initial reactants (lactose and lysine) are water soluble. However, as the reaction progresses some intermediate products are less water soluble, and indeed many of the final volatile compounds have limited water solubility. The partitioning of compounds between the aqueous and fat phases can impact the position of the equilibrium of any reaction by removing intermediates and/or products from the aqueous phase and into the fat phase. In particular, HMF is one component that has been shown to accumulate in the oil phase due to partitioning (23, 24).

Each compound has a partition coefficient (logP value) that is dependent on its structure (4, 25). Most logP values are obtained by measuring the amount of a compound partitioned in a hydrophilic phase, normally water, versus the amount partitioned into the hydrophobic phase, usually octanol (4). A positive logP value indicates that the compound partitions preferentially into a lipophilic phase. If the value approaches zero and becomes negative the compounds partition into the aqueous hydrophilic phase (4).

Across the two reaction systems the compounds that exhibit the largest changes in yield when fat was added are also those compounds that have a positive (lipophilic) logP value. The reaction mechanisms by which the compounds of interest are formed go through several intermediates, and analysis of their structure indicates that they would be expected to become more lipophilic as the reaction progresses along the mechanistic pathway to the final product (figure 5.14). If this is the

case, then the early reaction equilibrium may be driven by the intermediate products moving into the fat phase and pulling the equilibrium in favour of the product.

When fat is added to the system there is a large increase in maltol formation and an insignificant change in hydroxymaltol formation, suggesting that more of the intermediate products are forming in the presence of fat and that the intermediates are then forming higher quantities of maltol relative to hydroxymaltol. A hypothesised mechanism through a common intermediate is shown in figure 5.14 (26-28). This may be a partitioning effect due to the relative lipophilicity of maltol ($\log P = 0.090$) (29) when compared with hydroxymaltol ($\log P = -0.420$) (29) altering the equilibrium between these two compounds.

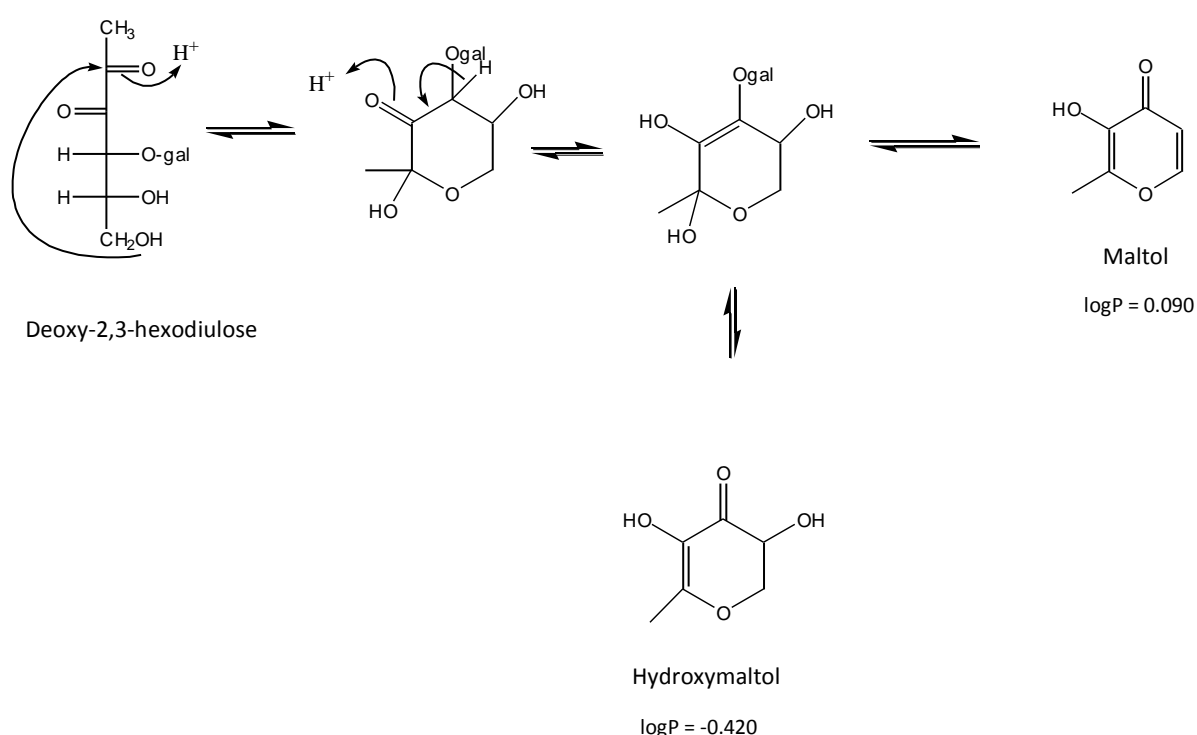


Figure 5.14 Schematic of the hypothesised pathways to hydroxymaltol and maltol via a common intermediate (26-28).

5.8 Conclusion

The presence of fat in the Maillard and caramelisation reactions affects volatile compound formation. The addition of fat to the reaction matrix containing the Maillard reaction model reactants generated an increase in all of the volatile compounds formed. The caramelisation reaction exhibited a less generalised increase, and only a few compounds were increased when fat was added. Since these volatile compounds indicate the potential for flavour within the system, this

implies that in a cooked food system fat can enhance flavour formation, and that a low fat food may contain fewer flavours formed during cooking. Therefore, when flavour formation during cooking is important to a food's finished flavour a reduction in fat can be detrimental.

Changing the added fat from canola oil (an unsaturated fat) to AMF (a saturated dairy fat) led to small changes in the final volatile compound profile for both the Maillard and caramelisation reactions. Relative to other influencing factors, such as the addition of fat and reaction type, the differences seen when the type of fat was changed from AMF to canola oil were statistically less significant. However, given the different flavour thresholds of the compounds, changing the type of fat could still generate a significant flavour difference in the final product. Further experiments would be required to fully explore how the individual components of fat influence the reactions.

The fat could be participating by regulating the temperature of the system and allowing reactants to maintain mobility by providing a liquid solvent environment after the water has evaporated. A correlation between increased reaction temperature and increased volatile compound formation was seen when cream and the aqueous reaction system were cooked and this could quite plausibly be responsible for some of the changes seen in the two phase system.

There was no evidence of any direct reaction occurring between any lipid oxidation products and amino acids. However, this does not rule out the possibility of other lipid oxidation product involvement at some point in the complex reaction cascade leading from reagents to final volatile products.

It was demonstrated that the flavours that were formed via the Maillard reaction mechanisms can be enhanced with the addition of fat: when a sugar based system where amino acids are not present, or are limited, the influence of fat was smaller. Some of the changes can be rationalised by considering partitioning effects between the oil and water phases. Based on these results, it can be concluded that the fat component of cream could have an important role in the generation of flavour in ghee. Whilst on the one hand increasing amounts of focus has been placed on low fat foods and on the other consumers are unwilling to compromise on flavour, serious consideration has to be given to the role the fat plays when foods are cooked.

5.9 References

1. Marangoni, A. G.; Acevedo, N.; Maleky, F.; Co, E.; Peyronel, F.; Mazzanti, G.; Quinn, B.; Pink, D., Structure and functionality of edible fats. *Soft Matter* **2012**, *8*, 1275-1300.
2. Van Ba, H.; Amna, T.; Hwang, I., Significant influence of particular unsaturated fatty acids and pH on the volatile compounds in meat-like model systems. *Meat Science* **2013**, *94*, 480-488.
3. Toyosaki, T., Sakane, Y., and Kasai, M., Effects of medium-chain triacylglycerols (MCT) on the Maillard reaction. *Journal of Food Processing and Technology* **2013**, *4*.
4. Leo, A.; Hansch, C.; Elkins, D., Partition coefficients and their uses. *Chemical Reviews* **1971**, *71*, 525-616.
5. Dunstan, T. S.; Fletcher, P. D. I., Compartmentalisation and separation of aqueous reagents in the water droplets of water-in-oil high internal phase emulsions. *Langmuir* **2011**, *27*, 3409-3415.
6. Holmberg, K., Organic reactions in microemulsions. *European Journal of Organic Chemistry* **2007**, 731-742.
7. Simonyan, G. S.; Beileryan, N. M., Michael condensation in a two-phase system: The application of inverse phase-transfer catalysis to the reaction of butyl acrylate with diethylamine in a water-heptane system. *Kinetics and Catalysis* **2002**, *43*, 337-340.
8. Przybylski, D. R., Canola Oil: physical and chemical properties. In Canola council of Canada: <http://www.canolacouncil.org/publication-resources/print-resources/technical-sheets/canola-oil-physical-and-chemical-properties/>, 2011; pp 1-6.
9. Edmondson, P. T.; Grammatika, M.; Fryer, P. J.; Handy, B., Modelling of heat transfer, mass transfer and flavour development in chocolate crumb. *Food and Bioproducts Processing* **2005**, *83*, 89-98.
10. Lioumbas, J. S.; Karapantsios, T. D., Evaporation front compared with crust thickness in potato deep-fat frying. *Journal of Food Science* **2011**, E17-E25.
11. Souza, E. C.; Fontan, R. C. I.; Verissimo, L. A. A.; Sampaio, V. S.; Silva, W. S.; Bonomo, R. C. F.; Veloso, C. M., Thermal diffusivity and specific heat of bovine milk affected by temperature and composition. *International Journal of Food Engineering* **2012**, *8*, 1-15.
12. Whitfield, F. B., Volatiles from interactions of Maillard reactions and lipids. *Critical Reviews in Food Science and Nutrition* **1992**, *31*, 1-58.
13. Ho, C. T.; Chen, Q. Y., Lipids in food flavours - An overview. In *Lipids in food flavors*, Ho, C. T.; Hartman, T. G., Eds. American Chemical Society: Washington, DC, United States of America, 1994; Vol. 558, pp 2-14.
14. Karademir, Y.; Goncuoglu, N.; Gokmen, V., Investigation of heat induced reactions between lipid oxidation products and amino acids in lipid rich model systems and hazelnuts. *Food and Function* **2013**, *4*, 1061-1066.
15. Potes, N.; Kerry, J. P.; Roos, Y. H., Oil as reaction medium for glycation, oxidation, denaturation, and aggregation of whey protein systems of low water activity. *Journal of Agricultural and Food Chemistry* **2013**, *61*, 3748-3756.

16. Pan, G. G.; Oliver, C. M.; Melton, L. D., alpha-dicarbonyl compounds formed by nonenzymatic browning during the dry heating of caseinate and lactose. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 6852-6857.
17. Pan, G. G.; Melton, L. D., Nonenzymatic browning of lactose and caseinate during dry heating at different relative humidities. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 10036-10042.
18. Ajandouz, E. H.; Desseaux, V.; Tazi, S.; Puigserver, A., Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry* **2008**, *107*, 1244-1252.
19. Parker, J. K., The kinetics of thermal generation of flavour. *Journal of the Science of Food and Agriculture* **2013**, *93*, 197-208.
20. van Boekel, M. A. J. S., Kinetic aspects of the Maillard reaction: a critical review. *Nahrung - Food* **2001**, *45*, 150-159.
21. Dumas, C.; Mittal, G. S., Heat and mass transfer properties of pizza during baking. *International Journal of Food Properties* **2002**, *5*, 161-177.
22. Severini, C. R.; Lerici, C., Interaction between the Maillard reaction and lipid oxidation in model systems during high temperature treatment. *Italian Journal of Food Science* **1995**, *7*, 189-196.
23. Morales, F. J.; Jimenez-Perez, S., HMF formation during heat-treatment of milk-type products as related to milkfat content. *Journal of Food Science* **1999**, *64*, 855-859.
24. Goncuoglu, N.; Gokmen, V., Accumulation of 5-hydroxymethylfurfural in oil during frying of model dough. *Journal of the American Oil Chemists Society* **2013**, *90*, 413-417.
25. Philippe, E.; Seuvre, A. M.; Colas, B.; Langendorff, V.; Schippa, C.; Voilley, A., Behavior of flavor compounds in model food systems: a thermodynamic study. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 1393-1398.
26. Kim, M. O.; Baltes, W., On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one in the Maillard reaction. *Journal of Agricultural and Food Chemistry* **1996**, *44*, 282-289.
27. Shaw, P. E.; Tatum, J. H.; Berry, R. E., 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one, A degradation product of a hexose. *Carbohydrate Research* **1971**, *16*, 207-213.
28. Yaylayan, V. A.; Mandeville, S., Stereochemical control of maltol formation in Maillard reaction. *Journal of Agricultural and Food Chemistry* **1994**, *42*, 771-775.
29. TheGoodScentsCompany, Flavours and fragrances. In <http://www.thegoodscentscompany.com/>, 2012.

Chapter 6 The role of emulsion structure in caramelisation and Maillard reactions

6.1 Introduction

Many food products, in particular dairy products, are emulsions (1). Cream and butter are both emulsions with different structures. Cream is an oil in water emulsion (o/w) where the oil droplets (discrete or dispersed phase) are suspended in the water (continuous phase). Butter is the reverse of this emulsion with a discrete phase of water and a continuous phase of oil to give a water in oil (w/o) emulsion (2). The generation of emulsions usually requires a combination of high shear, to create small droplets that are resistant to gravity separation, and an emulsifier that adsorbs at the newly created interface, providing a barrier against coalescence and imparting long term stability (2, 3).

In this chapter the addition of emulsion structure to the model system is investigated. Many model systems in literature use a thermodynamically stable emulsion, the microemulsion (4-12). Microemulsions display a number of properties different from those of conventional emulsions (2), including spontaneous formation, optical transparency and particular sensitivity to dilution and thermal conditions (13). These microemulsions usually require a high level of surfactant coupled with a low concentration of the dispersed phase. However, reactions within these microreactors can lead to unique products (5, 6, 11, 12). This chapter looks at whether the introduction of an emulsion structure can have a similar impact to the introduction of a microemulsion. This investigation will allow the influence of the cream emulsion structure to be assessed.

In a microemulsion, the extremely high surface areas and orientated surfactant layers provide many advantages to chemical reactions such as increased reaction rate, selective product formation, and lower energy input into the reaction system (4, 6-8, 11). This is particularly useful for reactions requiring extreme conditions, such as those involving two immiscible reactants or that contain compounds that are sensitive to temperature and environmental conditions (6). The phases of interest in microemulsions include discontinuous cubic phases that are optically isotropic, completely transparent and have a gel-like consistency, cubic phases that are a bicontinuous network of channels with a large interfacial area (12) and cubosomes (water dispersed cubic phases) (13).

Vauthy et al. (12) investigated structured fluids as microreactors for Maillard reaction chemistry. The reactions were carried out in aqueous, microemulsion and cubic phased reaction systems. The aqueous phase was 100% water, which was compared to a traditional w/o microemulsion with discrete and continuous phases. The cubic microemulsion is an interesting structure; it consists of channels of the two phases each of which is bicontinuous (12). When the reaction was carried out in the cubic phase there was a wider product profile along with increased product yields.

Garti and co-workers (4, 5) studied the model Maillard reaction in depth with a w/o emulsion stabilised with non-ionic surfactants. They looked at sucrose ester emulsions and compared the results with the aqueous phase with respect to water content within the microemulsion, reactant ratio, pH and temperature. With the generation of more than 200 volatile compounds (4) they simplified the system to use furfural rather than sugar; this limited the number of possible products. The results (4) showed two new compounds in the microemulsion system which were not present in the aqueous model. It was hypothesised that the environment at the interface and the concentration of reactants there may have lowered the activation energy required for this new reaction pathway to be available at the temperature of the reaction. The limiting factor for some of the reaction pathways is the solubilisation capacity (4) at the interfacial layer; this is unique to each reactant and interface interaction. A particular challenge in the use of microemulsions as microreactors in food systems is the difficulty in formulating a stable microemulsion using food grade materials, coupled with the need to use high levels of surfactant to accommodate the extremely high surface areas generated (5, 11).

In emulsion-based foods, it has been shown (4, 5, 7, 9, 12) that different emulsion structures can lead to different reaction product profiles. It is also known that ghee from different processes and from different parts of the world has different flavour profiles (14-18). Notably, the heating of butter or cream to make ghee generates a phase inversion of the emulsion. Phase inversions occur when the discrete phase and the continuous phase swap so that the discrete phase becomes continuous and the continuous phase becomes discrete (19). There are mechanisms by which this phase inversion can take place: catastrophic and transitional. A catastrophic phase inversion occurs when the dispersed phase becomes increasingly larger; inversion subsequently takes place abruptly causing the continuous phase to become engulfed by the coalescing droplets of the discrete phase. A catastrophic inversion will result in coalescing of the phases. Alternatively when the inversion is transitional a more gradual process occurs where intermediate stages occur with different structures. This process can be particularly important when an evaporation process is occurring (19) such as in the manufacture of ghee. In ghee the evaporation of water is a key part of the process

(chapter 3, section 3.2). In the initial stages of evaporation of water from cream the water to oil ratio was large, as the evaporation proceeded the ratio became increasingly smaller.

6.2 Formation of emulsions

The formulation of the emulsions was a crucial element of these experiments with the requirement to form stable emulsions with consistent droplet sizes from one emulsion to the next. Emulsions were generated using canola oil (rather than AMF) as it was easier to handle due to its liquidity at room temperature. This removed the complication that AMF adds to the emulsions when it begins to crystallise below 40 °C.

The hydrophilic lipophilic balance (HLB) of the emulsifiers provides an indication of what type of emulsion they are likely to form based on their structure (2). The small molecule surfactants commonly used as emulsifiers contain a hydrophobic group (often a long chain fatty acid) that can bind to the oil phase of an o/w emulsion and a hydrophilic head group that differs from emulsifier to emulsifier. The HLB provides an indication to the percentage contribution of the hydrophilic portion to the total weight of the emulsifier molecule (20).

Oil in water emulsions utilised Tween 60 (figure 6.1) as the emulsifier. Tween 60 (CAS 9005-67-8) is a polyethylene glycol sorbitan monostearate containing no unsaturated bonds and an HLB of 14.9 making it suitable for the formation of o/w emulsions (2). The Tween 60 molecule does not include groups likely to participate in Maillard or caramelisation reaction (unlike proteins that can also be used to stabilise o/w emulsions (21)) and has good stability against processing at higher temperatures, it is food grade and found commercially in various products. Polyglycerol polyricinoleate (PGPR) (figure 6.2) was used to generate the w/o emulsion. The PGPR is food grade with applications in chocolate, and widely used to create exceptional stability in w/o systems due to its low HLB value (~4) and structure (2).

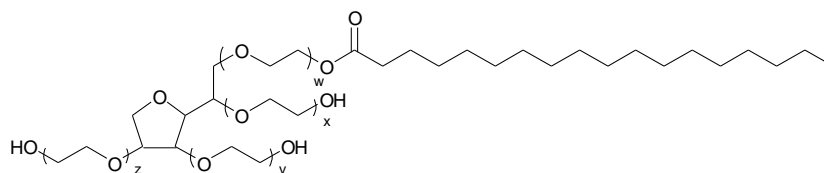


Figure 6.1 Polyethylene glycol sorbitan monostearate (Tween 60). Tween 60 is a group of isomers with the number of OCH_2CH_2 groups always equal to 20. $w + x + y + z = 20$ (2).

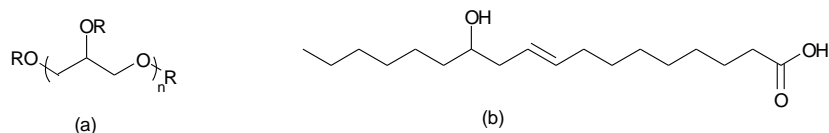


Figure 6.2 (a) Polyglycerol polyricinoleate in which R is hydrogen, ricinoleate acid or polyricinoleate acid. (b) Ricinoleate acid (3).

6.3 Impact of emulsifiers

The addition of the emulsifiers (Tween 60 and PGPR) to the matrix allowed the two different emulsion structures to be formed; however, any participation of these emulsifiers in the reactions or any chemical influence on reaction rate needed to be assessed. This was done using control samples (chapter 8, section 8.7.1) in which each of the emulsifiers was heated in a two phase solution, without an emulsion being formed, with the reaction components lactose, lysine or both. A blank sample of water and canola, the two phase reaction matrix, was cooked alongside the samples. These results are shown in figure 6.3, where only acetic acid was detected in the PGPR, Tween 60 and canola/water samples. The acetic acid was most likely due to the canola oil rather than any reactions taking place. Figure 6.4 compares the results achieved when lactose was cooked in the two phase matrix with the changes occurring when the emulsifiers were added to the lactose samples. The results of emulsifiers on lysine when cooked without lactose are shown in figure 6.5, while figure 6.6 demonstrates the effect of emulsifiers on the Maillard model containing lactose and lysine. These control samples were all run with phase ratios of 60% aqueous and 40% fat as per the two phase experiments (chapter 8, section 8.7.1).

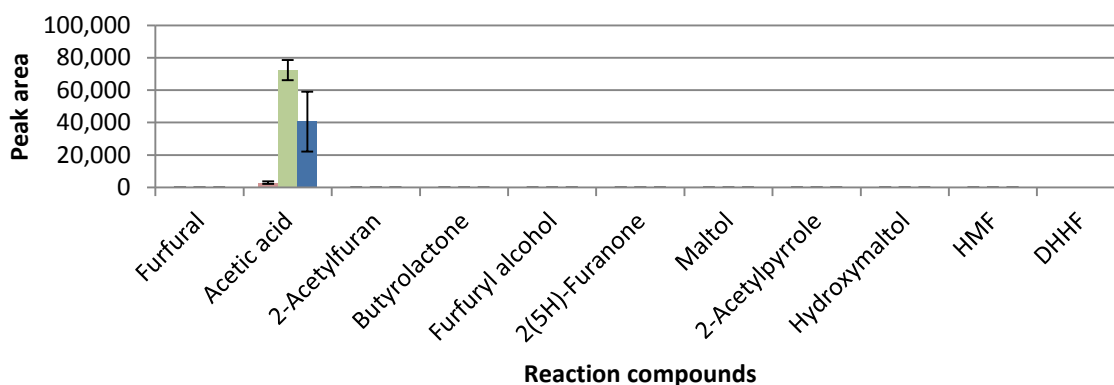


Figure 6.3 Baseline compound measurements after the heating of PGPR (red), Tween 60 (green) and canola/water (blue). The error bars represent the standard deviation of samples cooked in triplicate.

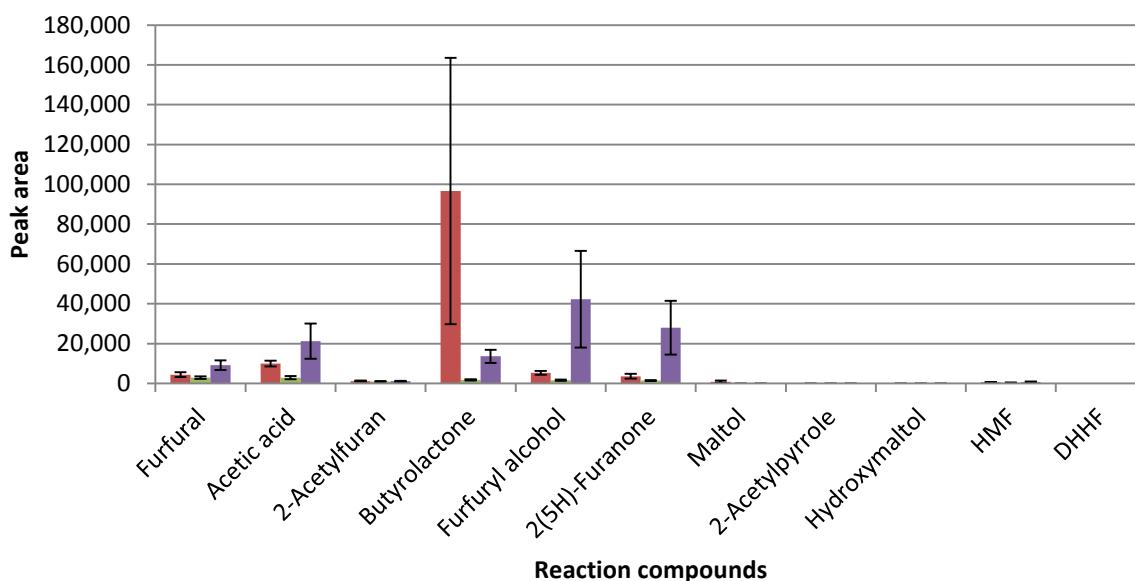


Figure 6.4 Influence of emulsifiers on the degradation of lactose in a two phase system: lactose only (red), lactose with PGPR (green) and lactose with Tween 60 (purple). The error bars represent the standard deviation of samples cooked in triplicate.

The large butyrolactone peak appears to be an anomaly when compared with previous caramelisation (lactose) experiments run under the same conditions (figure 4.1). Without considering the butyrolactone result there is a trend of PGPR reducing the peak area of the respective compounds while the Tween 60 appears to increase this peak area. Analytical control samples run during method development (section 2.11) indicate that these differences are not due to interference with the analytical method.

As expected none of the reaction compounds are formed when lactose is absent from the system (figure 6.5). A small amount of acetic acid is seen; however, this is a very small contribution to the overall results when compared to the results shown in figures 6.3, 6.4 and 6.6. The acetic acid was most likely from the canola oil.

There were some noticeable differences in results when the Maillard reaction model had different emulsifiers added (figure 6.6). There was a small change in compound formation when Tween 60 was added; however, there was a significant drop when the PGPR is added to the system. It could be speculated that the emulsifiers were forming micelles and crude emulsions during cooking due to the stirring of the samples, which were altering the results. Any differences between the two phase

systems and the emulsion systems need to take the emulsifier influences into account to determine the overall significance of any differences on the system.

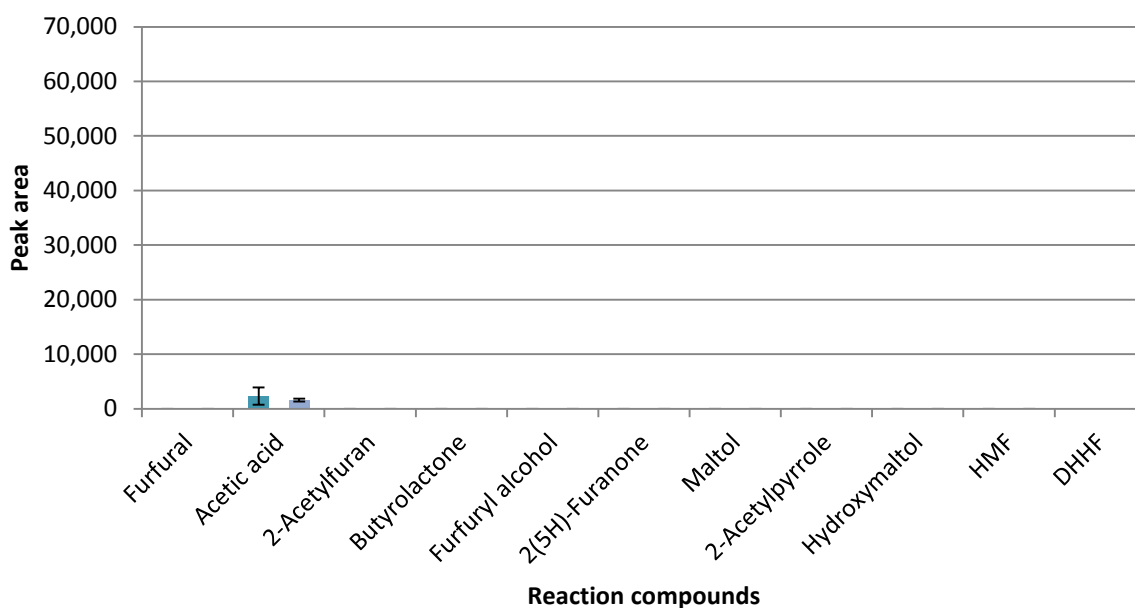


Figure 6.5 Interactions between lysine and emulsifiers in a two phase system: lysine (teal), lysine with PGPR (orange) and lysine with Tween 60 (blue). The error bars represent the standard deviation of samples cooked in triplicate.

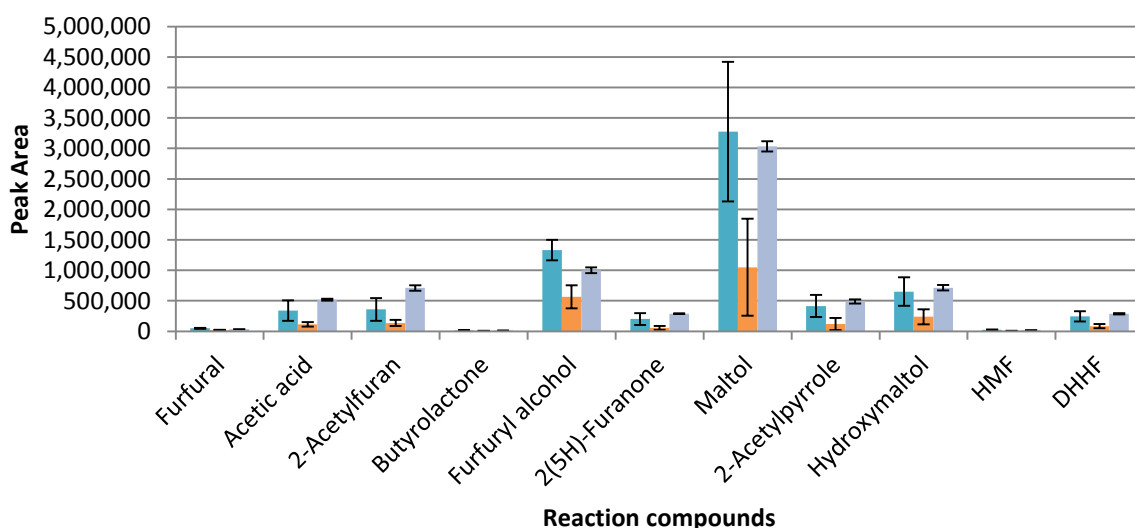


Figure 6.6 Influence of the emulsifiers on the Maillard reaction model (lactose and lysine): lactose and lysine (teal), lactose and lysine with PGPR (orange), lactose and lysine with Tween 60 (blue) were all cooked in triplicate using a two phase system. The error bars represent the standard deviation of triplicate samples.

6.4 Influence of emulsion structure

The two emulsifiers were used to generate o/w and w/o emulsions as described in chapter 8, section 8.7. These two emulsion structures generate two different distributions of the reactants (lactose and lysine). In the o/w emulsion the reactants are present in the continuous phase whereas in the w/o emulsion, the reactants are contained within in the oil in discrete droplets of water. Due to time constraints on the large number of experiments required to fully explore the emulsion system the Maillard reaction received the most focus with repeated experiments; the caramelisation reactions were only carried out once with duplicate samples cooked.

The control testing (figure 6.6) indicated that some small differences in product formation could be expected when the Maillard reaction was occurring between lactose and lysine in the presence of Tween 60. Therefore, the control sample containing Tween 60 was used to determine if there was any further influence (other than the presence of the Tween 60) on the Maillard reaction when the emulsion structure was generated through high pressure homogenisation (2). A comparison of the Maillard reaction occurring in a two phase system containing Tween 60 and the o/w emulsion (figure 6.7) shows a difference in the formation of some volatile compounds being achieved when the reaction was occurring in an o/w emulsion structure.

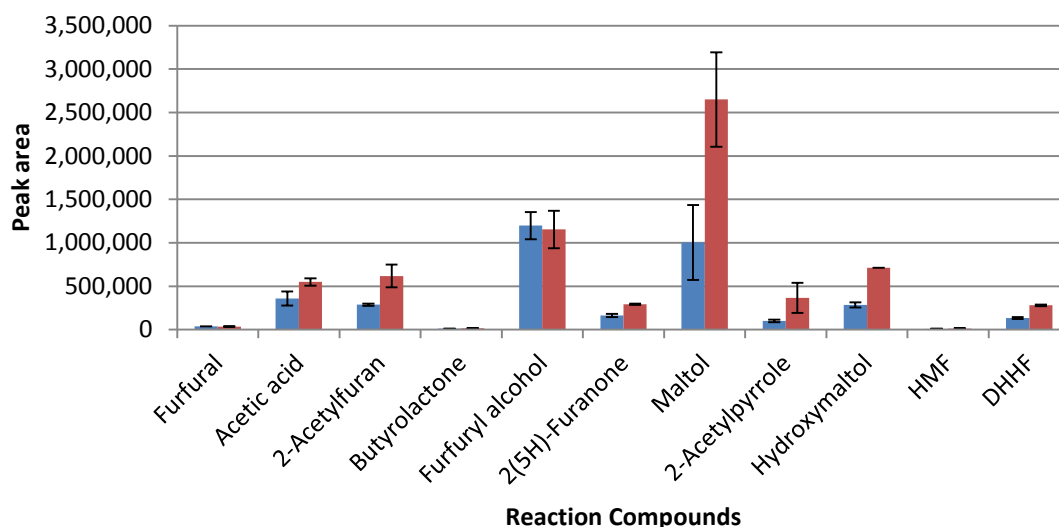


Figure 6.7 Maillard reaction compounds formed in the o/w emulsion (blue) compared with the two phase control sample containing Tween 60 (red). The error bars represent the standard deviation of triplicate samples.

The o/w structure of the emulsion is the same structure that cream exhibits and ANOVA results (appendix C, table C7) (significance $\alpha=0.05$) indicated significant differences for that 2-acetylfuran

($p=0.01$), 2-acetylpyrrole ($p=0.02$) and hydroxymaltol ($p=0.05$) as compared with the two phase structure containing Tween 60 when the o/w emulsion structure was introduced. Both maltol and DHHF had p values that were below 0.1 but above the 0.05 significance level. These compounds all decreased in yield with the emulsion structure addition.

One of the emulsion parameters altered was the droplet size (table 6.1). The parameters measured included the surface area weighted mean ($D[3,2]$) which is the average surface area (calculated) of the droplets, $d(0.1)$, $d(0.5)$ and $d(0.9)$ represent the maximum droplet diameter that 10%, 50% and 90% of the droplets measure respectively (22). The volume weighted mean ($D[4,3]$) can also be calculated and represents the mean volume of a droplet (22). In the case of the o/w emulsion this increased the surface area of oil within the emulsion as the oil droplets were made smaller through higher pressure processing. Cream had a measured droplet size of $D[3,2] = 0.6$ (averaged across two cream samples) and a droplet diameter range from $d(0.1) = 0.2$ to $d(0.9) = 7.0 \mu\text{m}$. 10% of cream droplets have a diameter less than or equal to $0.16 \mu\text{m}$ and 90% of the droplet diameters fall below $7.3 \mu\text{m}$. Using different pressure settings on the homogeniser and different speed settings on the ultraturrax, a range of droplet sizes were formed to explore the impact of droplet size may have on the reactions (figure 6.8). These droplet sizes could be replicated across different emulsions using the same homogeniser settings (figure 6.8); other droplet size parameters such as volume weighted mean and surface weight mean were also measured (table 6.1) to gauge not only the diameter of the droplets but also the droplet volume and surface area.

Table 6.1 Droplet size (μm) parameters for emulsion samples (values are the average of duplicate samples, each measured twice)

Sample Name	$D[4, 3]^*$	$D[3, 2]^*$	$d(0.1)$	$d(0.5)$	$d(0.9)$
Maillard homogenised	0.6	0.4	0.2	0.5	1.0
Maillard homogenised	1.0	0.5	0.2	0.6	2.0
Maillard UT20000	10.0	1.0	1.0	9.0	19.0
Maillard UT20000	9.0	2.0	1.0	8.0	17.0
Maillard UT13500	15.0	8.0	6.0	14.0	26.0
Maillard UT13500	15.0	6.0	2.0	14.0	27.0
Maillard UT8000	41.0	34.0	21.0	37.0	66.0
Maillard UT8000	34.0	14.0	15.0	33.0	56.0

* $D[4, 3]$ - Volume weighted mean; $D[3, 2]$ - Surface weighted mean

Each of these emulsion samples was cooked to generate volatile compounds to explore whether the manipulation of droplet size could lead to a significant change in the results. The results are shown

in figure 6.9 and represent the average peak areas formed across the two experiments (chapter 8, section 8.7.2).

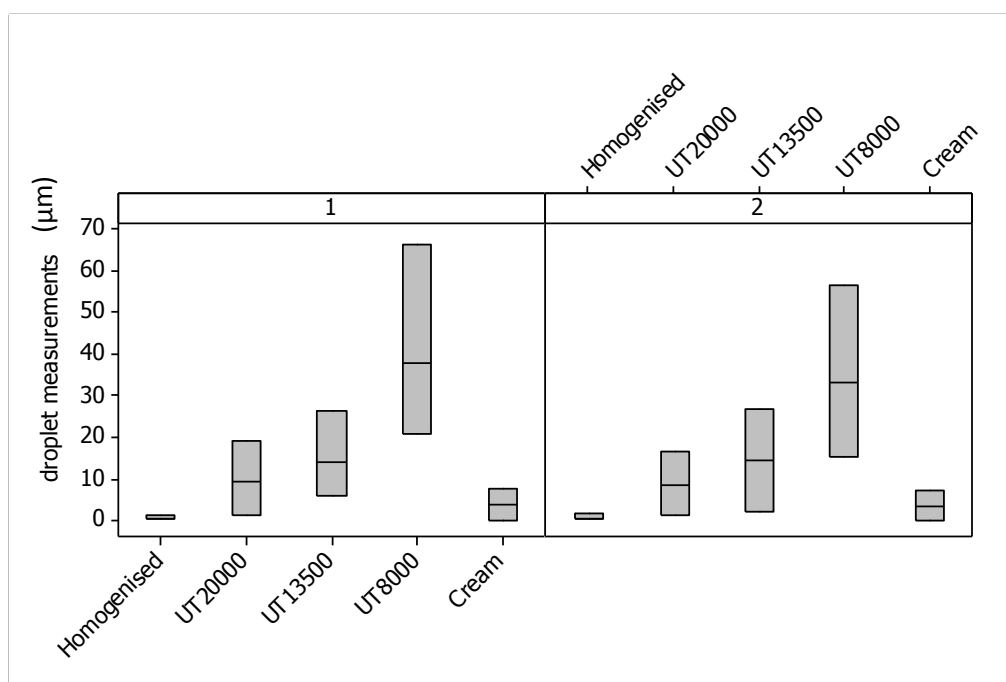


Figure 6.8 The range of droplet sizes achieved across two experiments as compared to the droplet sizes of cream. The bars of each box represent the d(0.1), d(0.5) and d(0.9) respectively from the bottom of each box up.

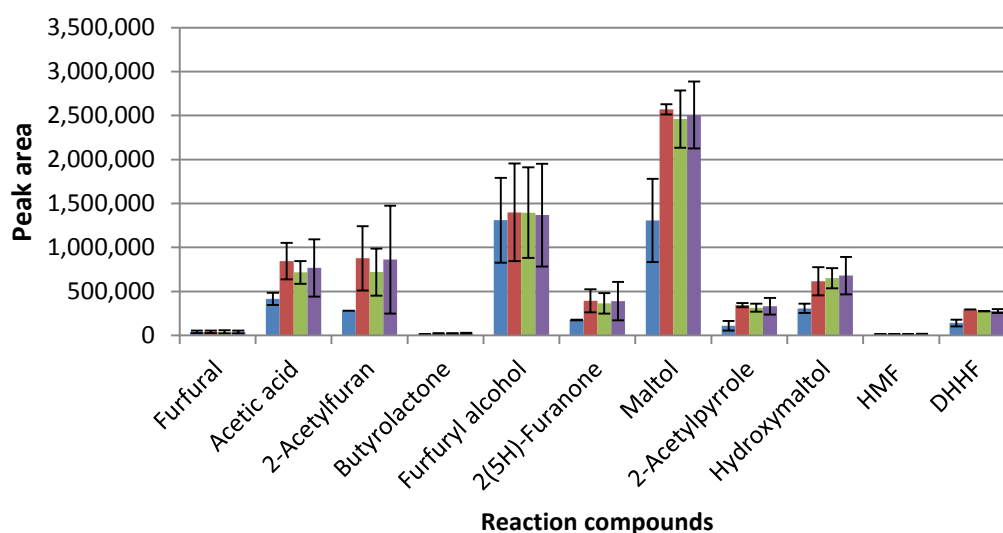


Figure 6.9 Averaged data showing the influence of droplet size on the Maillard compounds formed in an oil in water emulsion structure. Droplet sizes were generated using the ultraturrax at 20,000 rpm (red), 13,500 rpm (green), 8,000 rpm (purple) and the homogeniser (blue). The error bars represent the standard deviation of triplicate samples from two experiments.

The addition of the emulsion structure similar to that of cream in a range of droplet sizes yielded a varied influence on the Maillard reaction with some significant impact (appendix C, table C8) on the formation of volatile compounds (figure 6.9). The smallest droplet size as generated through homogenisation appeared to have generated a lower level of compound formation relative to the larger droplet sizes. However, amongst the larger droplet sizes there were no significant differences in compound formation. When the compound formation for the larger droplet sizes is compared with that of the two phase control sample that contains the emulsifier there was no significant difference (figure 6.10). This could suggest that the emulsion structure coalesced at a faster rate when the larger droplet sizes were used resulting in a structure similar to that of the two phase system during cooking.

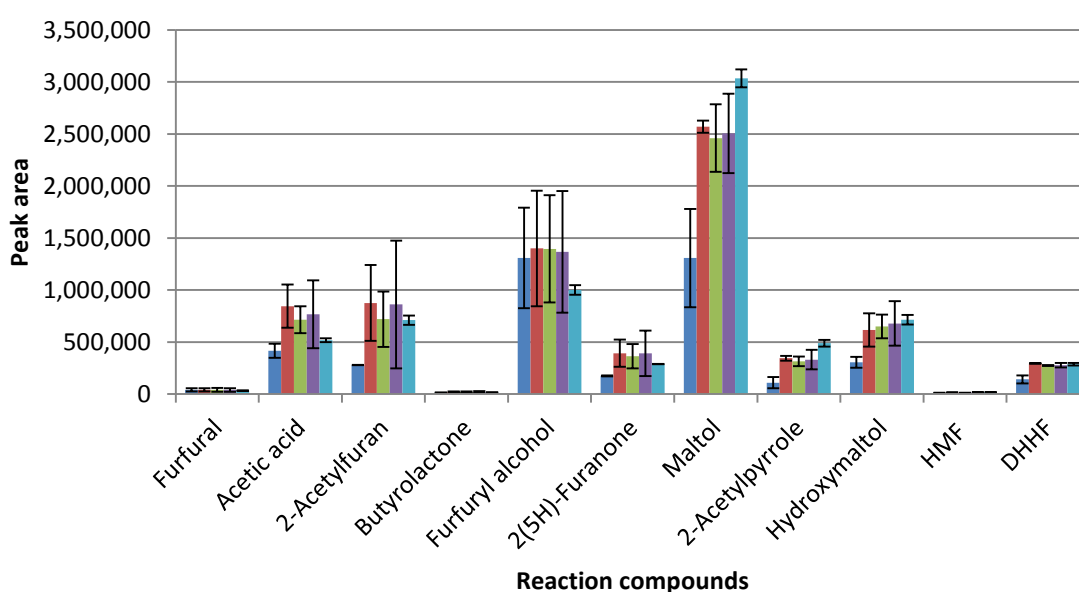


Figure 6.10 Averaged data showing the influence of droplet size on the Maillard compounds formed in an oil in water emulsion structure. Droplet sizes were generated using the ultraturrax at 20,000 rpm (red), 13,500 rpm (green), 8,000 rpm (purple), the homogeniser (blue) and the two phase Tween 60 control sample (light blue). The error bars represent the standard deviation of triplicate samples from two experiments.

The impact of the addition of the emulsion structure and the different droplet sizes on the caramelisation reaction can be seen in figure 6.11, the droplet sizes are given in table 6.2; comparison with the average Maillard experiment droplet sizes are given in small italics.

Table 6.2 Droplet sizes (μm) for the caramelisation experiment compared to the average Maillard reaction droplet sizes (*italics*) (values are the average of duplicate samples, each measured twice)

Samples	d(0.1)	<i>d(0.1)</i>	d(0.5)	<i>d(0.5)</i>	d(0.9)	<i>d(0.9)</i>	D[4,3]*	<i>D[4,3]</i>
UT8000	25.0	<i>18.0</i>	42.0	<i>35.0</i>	67.0	<i>61.0</i>	44.0	<i>38.0</i>
UT13500	10.0	<i>4.0</i>	19.0	<i>14.0</i>	32.0	<i>27.0</i>	20.0	<i>15.0</i>
UT20000	3.0	<i>1.0</i>	10.0	<i>9.0</i>	20.0	<i>18.0</i>	11.0	<i>9.0</i>
Homogeniser	0.2	<i>0.2</i>	0.5	<i>0.5</i>	1.0	<i>2.0</i>	0.6	<i>0.8</i>

*D [4, 3] - Volume weighted mean

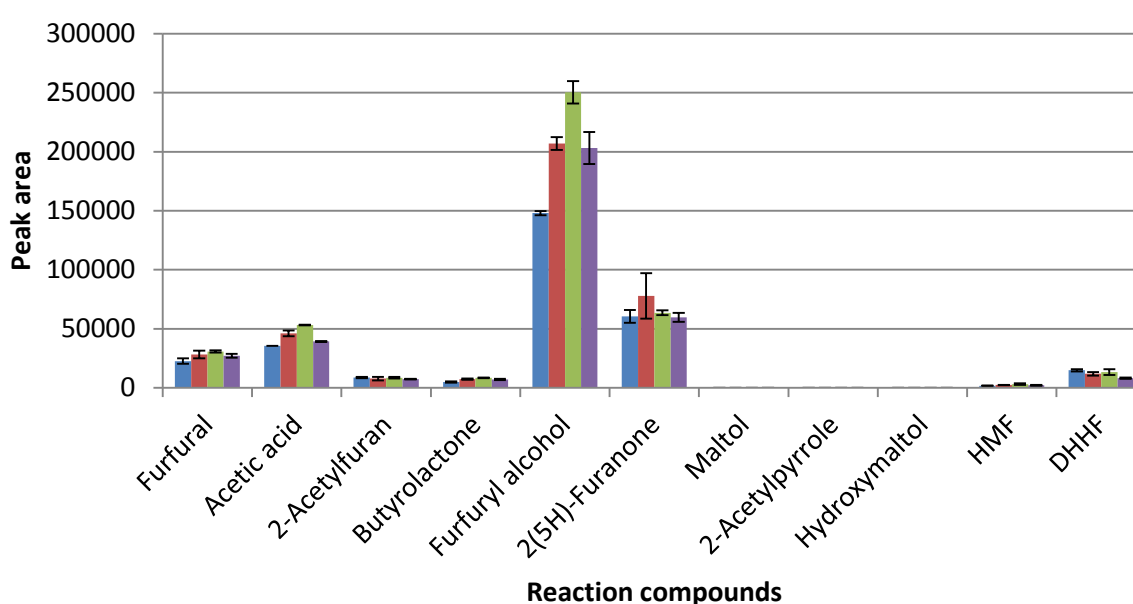


Figure 6.11 The impact of emulsion structure (o/w) and droplet size on the caramelisation reaction. The droplet sizes were generated using the ultraturrax at 20,000 rpm (red), 13,500 rpm (green), 8,000 rpm (purple) and the homogeniser (blue). The error bars represent the standard deviation of duplicate samples.

There were some significant differences in the caramelisation reaction when the emulsion was designed as per cream (o/w emulsion) (appendix C, table C9) and manipulated for droplet size (table 6.1 and 6.2) (appendix C, table C10). In the control samples (figure 6.4) an increase in compound formation was seen upon addition of the Tween 60 emulsifier, when the emulsion structure was added a further increase was seen in some compounds, namely furfuryl alcohol and acetic acid (figure 6.12). The significance of these increases could lead to potential flavour changes in a caramelisation reaction; however, this is only a small portion (~10%) of the compounds total value as

generated by the Maillard reaction system and so is unlikely to have any impact on flavour when the caramelisation reaction is occurring alongside the Maillard reaction.

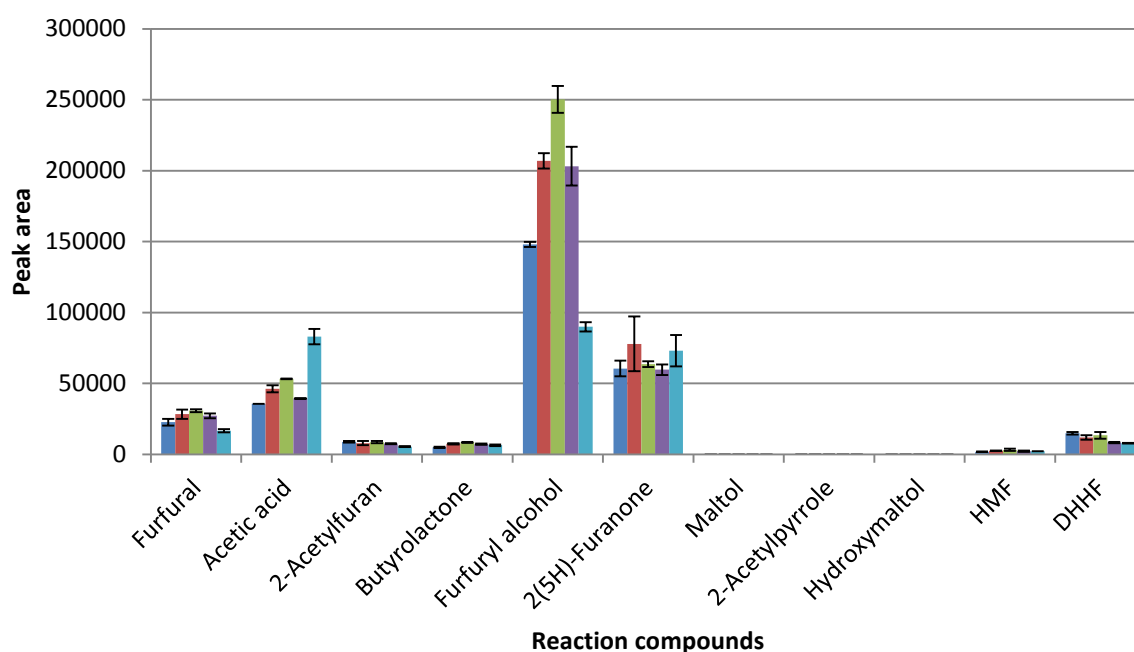


Figure 6.12 The impact of emulsion structure (o/w) and droplet size on the caramelisation reaction. The droplet sizes were generated using the ultraturrax at 20,000 rpm (red), 13,500 rpm (green), 8,000 rpm (purple) and the homogeniser (blue). The two phase control sample contains Tween 60 (light blue). The error bars represent the standard deviation of duplicate samples.

An inversion of the o/w emulsion structure to the w/o emulsion structure was predicted to generate a more significant change as the dynamics of evaporation and heat transfer could be altered by forming discrete water droplets within the oil phase. As seen previously (section 6.3) the addition of PGPR to form the w/o emulsion generated a drop in the formation of volatile compounds; when this system was homogenised to form small water droplets within the oil phase an increase in all compound formation was seen. These results are shown in figure 6.13.

There was an increase in many of the compounds formed by the Maillard reaction (figure 6.13) when the emulsion structure was formed to generate discrete droplets of water inside the continuous oil phase compared with the two phase control solution containing no emulsion structure. When this was compared with the results seen for an o/w emulsion and also a standard two phase system without emulsifiers the differences achieved can be seen (figure 6.14).

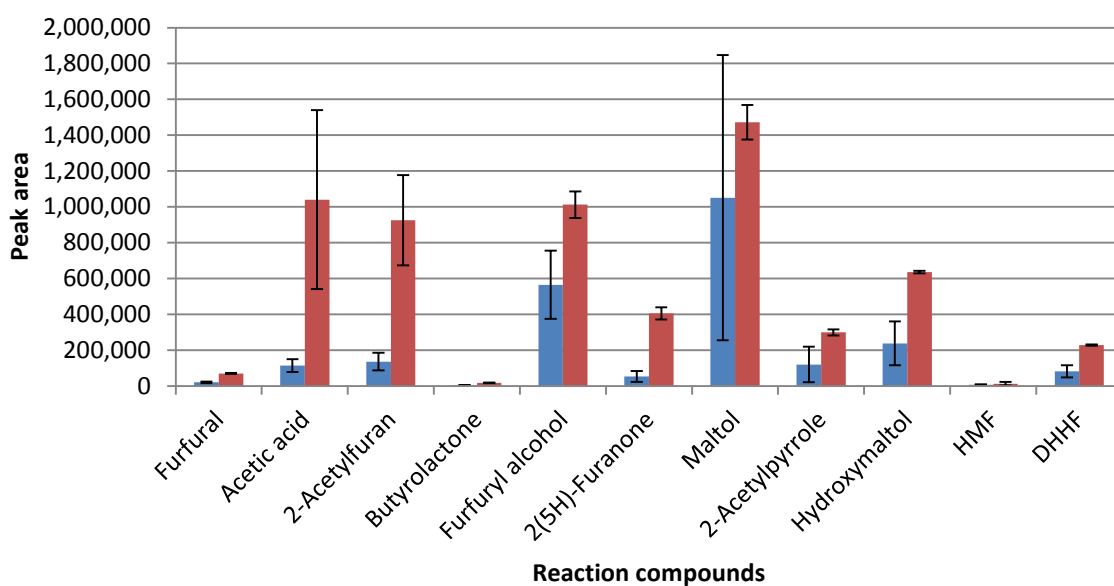


Figure 6.13 The Maillard reaction in a w/o emulsion (red) compared with a control solution of reactants and emulsifier with no emulsion structure (blue). The error bars represent the standard deviation of duplicate samples across two experiments.

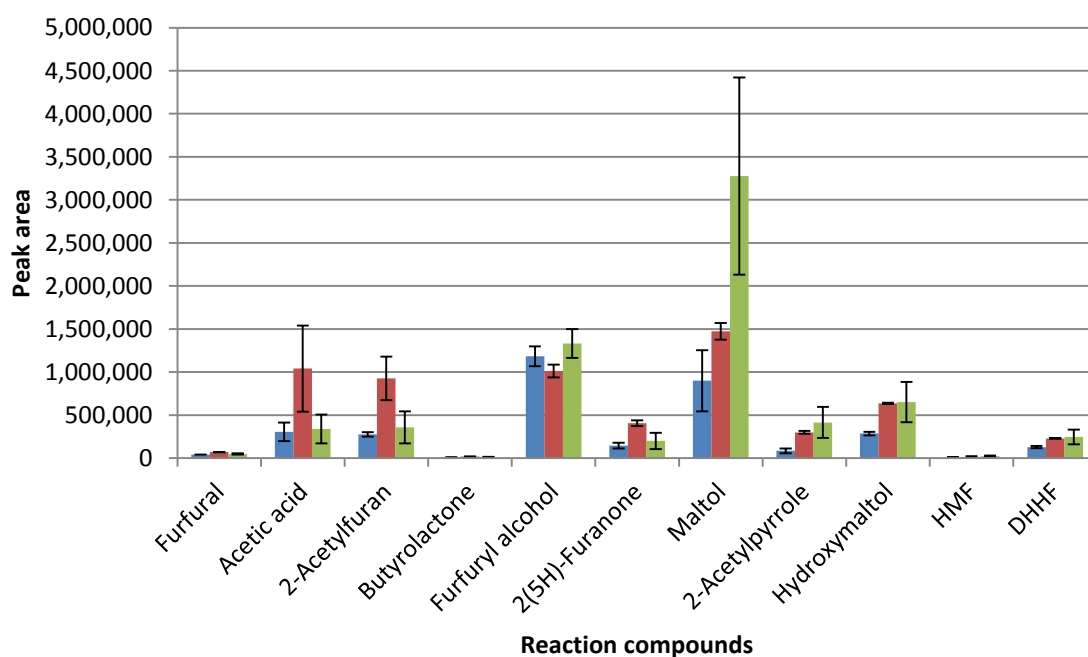


Figure 6.14 Comparison of the emulsion structures (o/w, blue; w/o, red) with a two phase sample containing no emulsion structure or emulsifiers (green). The error bars represent the standard deviation of duplicate samples across two experiments.

The comparison of results gained through the different emulsion structures (figure 6.15) indicates that different flavours could be formed using different emulsion structures. The w/o emulsion generates a higher level of many flavours compared with the o/w emulsions. However, when these levels are compared with the two phase system (figure 6.14) there are only some compounds that show an increased level of volatile compound formation: acetic acid, 2-acetylfuran and 2(5H)-furanone. The only compound to have decreased with the introduction of the emulsion structure was maltol; however, with the large error present for the two phase system it was difficult to conclude how significant this decrease was.

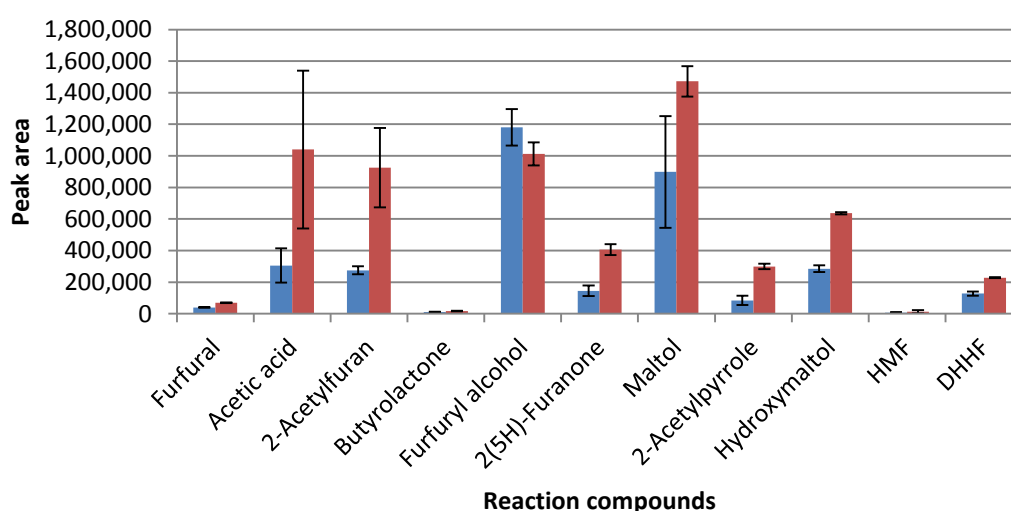


Figure 6.15 The direct comparison of the two emulsion structures influence on the Maillard reaction: w/o emulsion structure given in red and o/w emulsion structure given in blue. The error bars represent the standard deviation of duplicate samples across two experiments.

The results for the Maillard reaction system were significantly different when the emulsion structure was inverted to a w/o emulsion (appendix C, table C11). However, when the caramelisation reaction model was put into the inverted emulsion (figures 6.16 and 6.17) few differences were seen, with the exception of an increase in the 2-acetylfuran and 2(5H)-furanone peaks. The formation of 2-acetylfuran decreased with the introduction of the w/o emulsion structure while there was an increase in the formation of 2(5H)-furanone (figure 6.16).

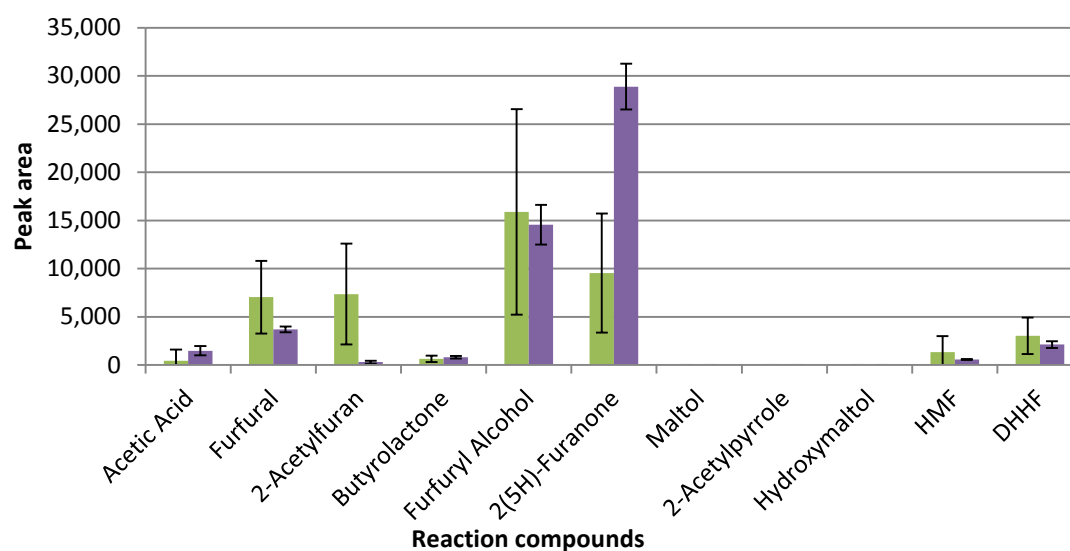


Figure 6.16 The caramelisation reaction in a w/o emulsion (purple) as compared to a two phase system containing PGPR (green). Error bars represent the standard deviation of duplicate samples.

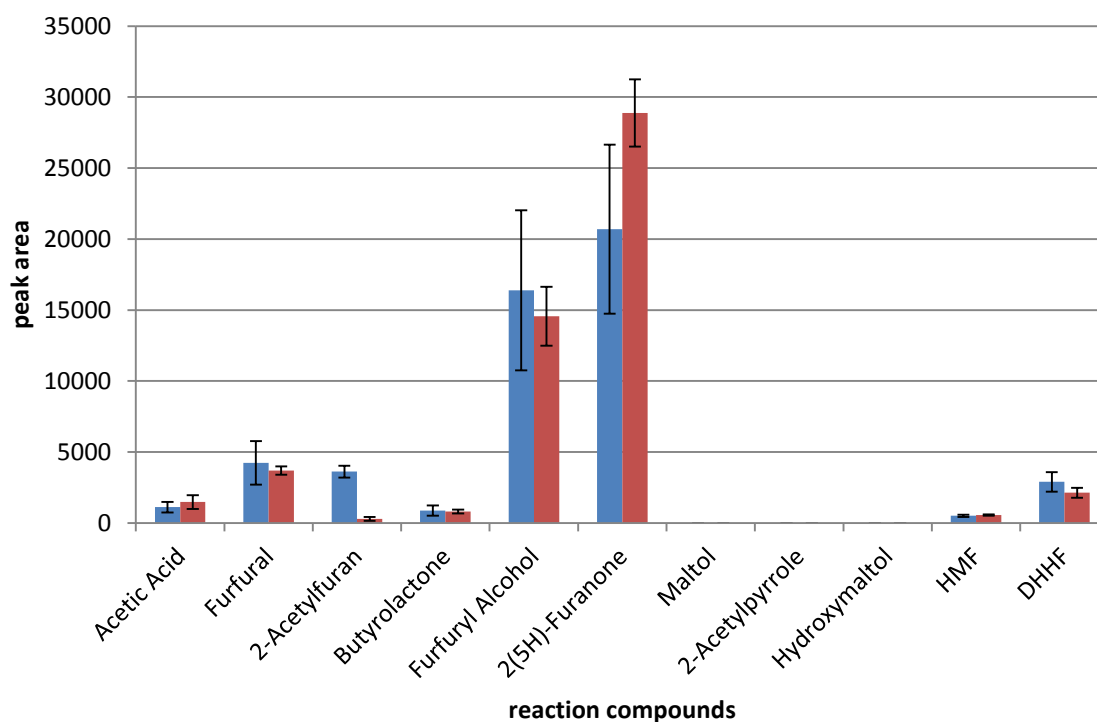


Figure 6.17 Direct comparison of the influence that the emulsion structure has on the caramelisation reaction (o/w, blue; w/o, red). Error bars represent the standard deviation of duplicate samples.

Given that there was a difference seen in the volatile compound results between the o/w emulsion and the w/o emulsion, a set of experiments was run to look at the changes over time in conjunction with the temperature and evaporation of the reaction system. Figure 6.18 shows the changes in volatile compounds over the 20 min cooking time, each sample was cooked for two minutes longer than the previous sample. Over the cooking time the observation of colour changes within the samples was made. The o/w samples became brown as the emulsion separated around 15-16 minutes and continued to develop colour until the end of cooking. The final o/w samples were a clear oil phase with a dark brown solid cooked onto the bottom of the vial. In contrast, the w/o samples contained dispersed dark brown solid particles at the end of the cooking process; colour development started to occur from 12-14 minutes.

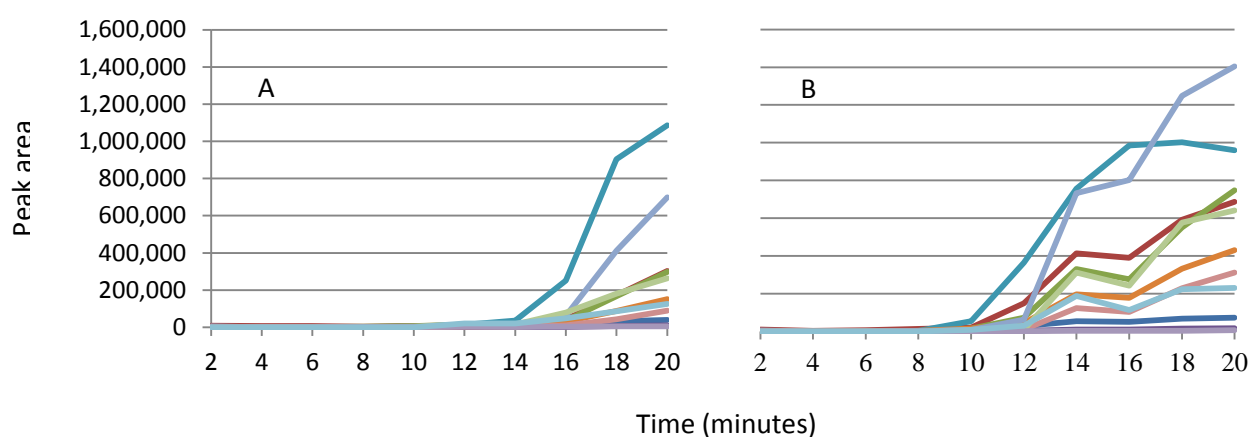
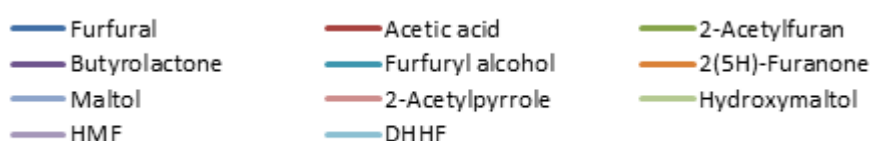


Figure 6.18 (A) Maillard reaction o/w emulsion cooked in time series over two minute intervals. (B) Maillard reaction w/o emulsion cooked in time series over two minute intervals.



There were two other differences that were noticed when the time series experiments were run that could help to explain why the difference was seen in the Maillard reaction. Past results (chapter 5) had shown that time and temperature, are both very important to the rate of the Maillard reaction and that different compounds have different rates of reaction. Figure 6.18 shows the average data for the temperature changes with the emulsions and figure 6.19 the rate of evaporation over the course of a 20 minute reaction.

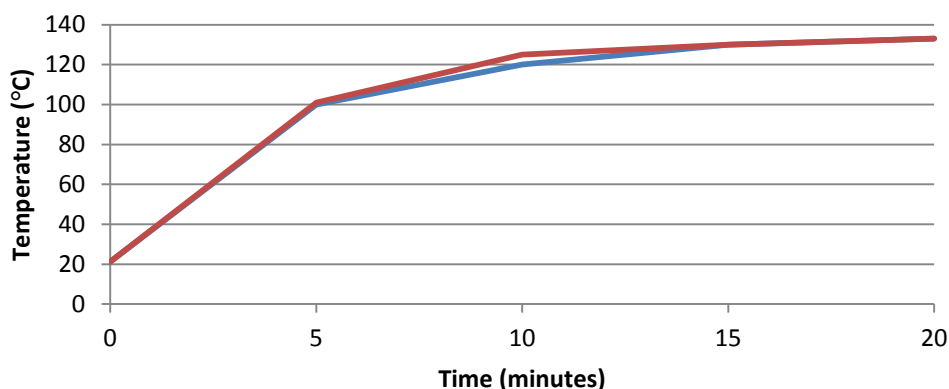


Figure 6.19 The internal sample temperatures of the two different emulsion structures: w/o emulsion is given by the red line and the o/w emulsion is given by the blue line.

There was no significant difference in temperatures within the emulsion samples although the measurements were difficult to make given the small amount of sample present. A larger scale reaction with real time monitoring of the samples could provide a more useful insight into the internal temperature of the samples. Likewise, the rate of evaporation (figure 6.20) across the two samples indicated only a small difference in overall rate and may not be significant enough to cause the differences seen between the two emulsion structures. Further investigation into the influence of evaporation rates could shed some light on the impact of these differences.

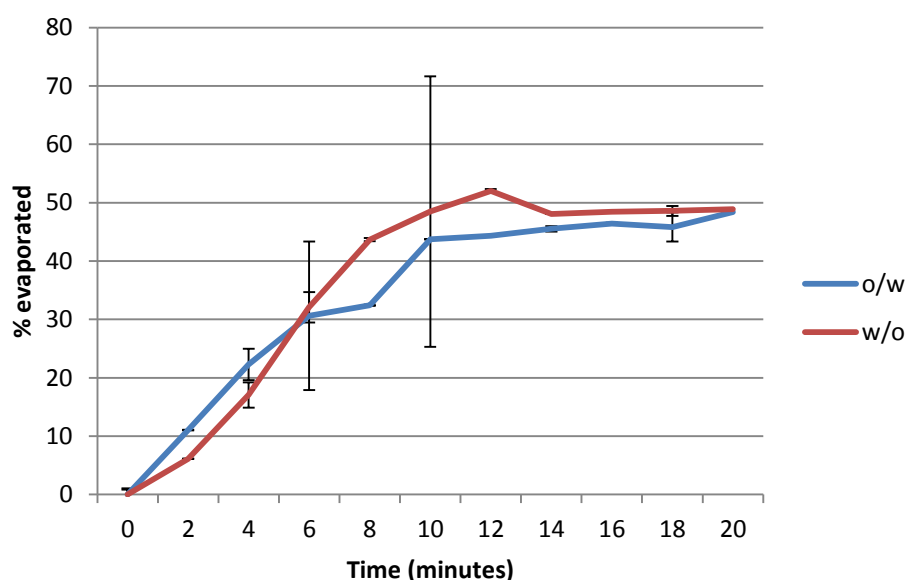


Figure 6.20 The rate of evaporation for the different emulsion structures: w/o emulsion given in red; o/w emulsion given in blue. Error bars represent the standard deviation of triplicate samples.

The measurements of temperature and evaporation rate provided no measureable difference within the error between the two emulsion structures during cooking and are therefore unlikely to be sufficient enough to generate the differences seen. Changes over time of the internal emulsion structure along with the fundamental differences between the structures could contribute to the differences in results.

To investigate the changes occurring in the emulsion structures during cooking, a microscopy experiment was set up to capture images (chapter 8 section 8.7.6) of the emulsion structure at 2 minute intervals. The microscopy images (figure 6.21) provide a time stamped guide to what was occurring during the cooking of the emulsions. Samples of the Maillard reaction in an o/w and w/o emulsion were made up as per chapter 8 section 8.7.2 and cooked 0 – 20 minutes at 2 minute time intervals. At the end of each two minute time interval a tiny droplet of the sample was removed from the vial and placed on a microscope slide with a cover slip. Images were taken of the samples and compared to identify the changes in emulsion structure that were taking place during cooking.

Early in the cooking process (time = 2 minutes) both emulsions have internal temperatures less than 100 °C (figure 6.18). The w/o emulsion consists of water droplets in oil and the o/w emulsion is oil droplets in water. The droplets in both emulsions are coalescing. Further on in the cooking process as the cooking time approaches 10 minutes with both emulsions reaching internal temperatures in excess of 100 °C (figure 6.18) with the w/o emulsion exhibiting a slightly higher temperature than the o/w emulsion. The water has mostly evaporated from the w/o emulsion, the o/w emulsion structure has become highly coalesced. At 12 minutes a faster rate of evaporation has occurred in the w/o emulsion with the o/w emulsion containing a higher % of water. Development of colour can clearly be seen in the w/o emulsion and the structure of the o/w emulsion continues to collapse. The detection of volatile flavour compounds in the w/o emulsion exceeds those detected in the o/w emulsion. After 14 minutes of cooking time the colour development continues in the w/o emulsion becoming increasingly dark brown with o/w emulsion fully separated and some colour formation beginning. This colour development corresponds with an increase in volatile compound formation. At the end of the cooking process (20 minutes) dark droplets (melanoidins) are seen suspended in the w/o emulsion (A) whereas the o/w emulsion (B) has no suspended particles or droplets. The profile of volatile compounds differs between the two emulsions.

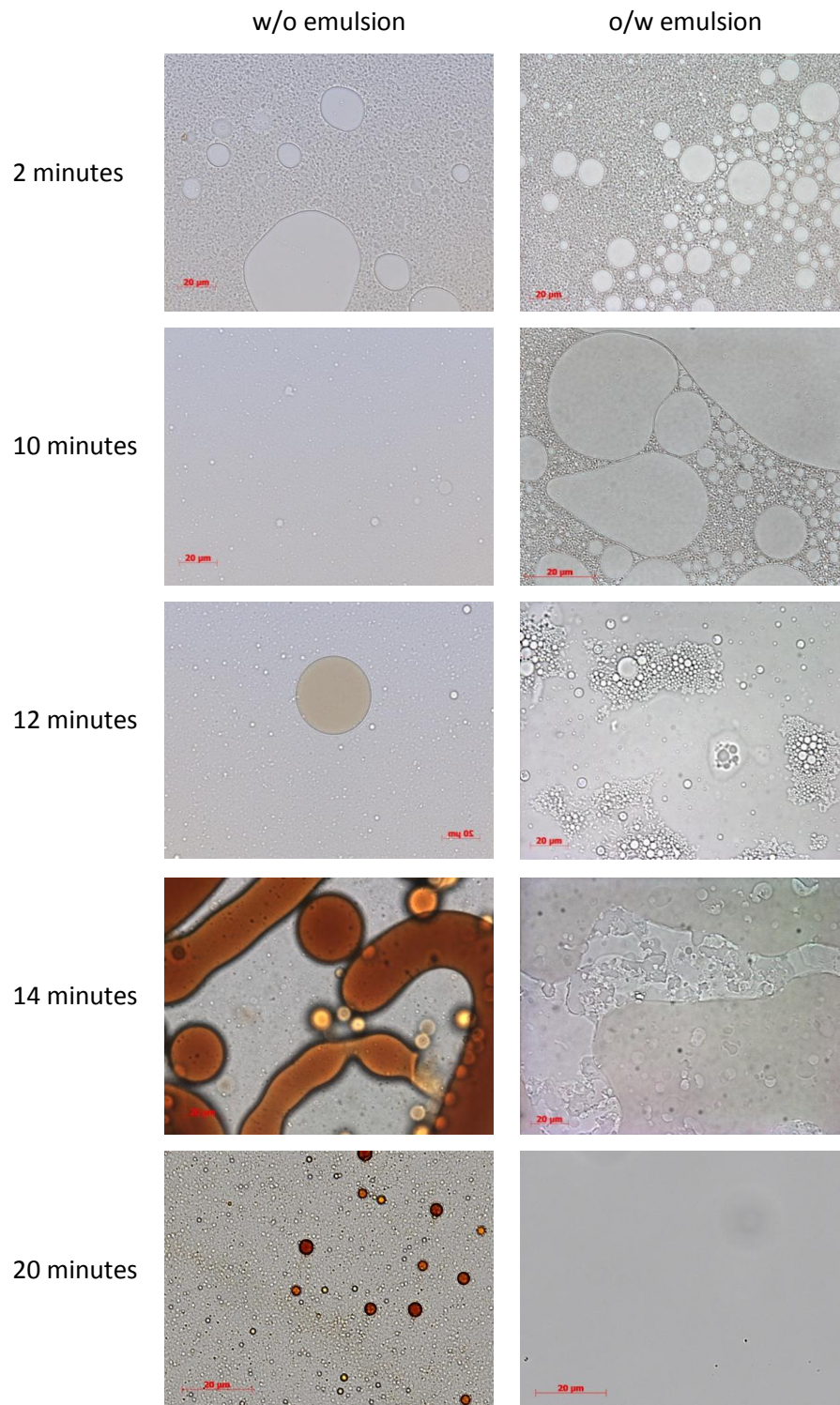


Figure 6.21 Microscopy images of the structural changes occurring in w/o and o/w emulsions during cooking.

The microscopy images show clearly the coalescing of the droplets early in the cooking process; this provides a possible explanation as to why the changes in droplet size did not lead to a significant difference for the o/w emulsion results. The colour development as discussed previously begins at

12 minutes for the w/o emulsion and the colour particles are visible throughout the samples until the end of cooking. The o/w emulsion displays no colour development until closer to 14 minutes and the particles are not suspended in solution so were not sampled for the microscopy. This is congruent with the observations of the sample vials and colour changes during the cooking process. The catastrophic inversion (separation) of the o/w emulsion between 8 and 12 minutes can be seen clearly in the microscopy images while the w/o emulsion continues to shrink the droplet sizes as the water evaporates and does not appear to undergo the same transitional separation as the o/w emulsion. A future experiment that could be interesting would be to investigate the relative stabilities of different emulsions and control the point at which they invert or maintain their structure throughout the cooking to control the Maillard reaction.

6.5 Conclusion

The introduction of the emulsion structure to the model reaction system led to some changes within the reaction systems, although not all of the changes translated into changes in volatile compound profiles. The interactions of emulsifiers with the reactants provided some interesting results although they were not the focus of this study; they were used as control samples. It could be hypothesised that the addition of the emulsifiers allowed micelles to form, which in turn influenced the partitioning of the reactants, intermediate products and final products leading to the altered results. In contrast when the emulsion structure was added there was little change in the o/w emulsion compared with the control sample and compared with the two phase samples discussed in chapter 5. In future experiments, the stability of the emulsion and type of emulsifier along with the amount of emulsifier could be altered in an effort to shift the reaction.

The use of microemulsions as microreactors (4-6, 9-12) for the Maillard reaction led to the formation of higher yields of volatile compounds and different compounds forming; however, this same phenomenon was not seen in the emulsions based on food products under these conditions. The Maillard reaction exhibited significant changes in the formation of all compounds with the change in emulsion structure, but the caramelisation reaction only presented significant changes in the formation of two compounds formation when the emulsion structure was changed. The greatest level of change was seen when the emulsion structure was altered from an o/w structure to a w/o structure. The change in compound formation using the w/o emulsion structure generated a change in the ratio of the compounds rather than just increasing the other all level of formation as was seen when fat was added to the reaction (chapter 5). The ability to generate different volatile compound profiles between the o/w and w/o emulsions could lead to the generation of different flavour profiles when applied to food design.

6.6 References

1. TetraPak, *Dairy processing handbook*. Tetra Pak Processing Systems: Lund, Sweden, 1995.
2. Friberg, S. E.; Larsson, K.; Sjoblom, J., *Food emulsions*. Marcel Dekker Inc: New York, NY, United States of America, 2004.
3. Ushikubo, F. Y.; Cunha, R. L., Stability mechanisms of liquid water-in-oil emulsions. *Food Hydrocolloids* **2014**, *34*, 145-153.
4. Fanun, M.; Leser, M.; Aserin, A.; Garti, N., Sucrose ester microemulsions as microreactors for model Maillard reaction. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2001**, *194*, 175-187.
5. Garti, N., Microemulsions as microreactors for food applications. *Current Opinion in Colloid and Interface Science* **2003**, *8*, 197-211.
6. Holmberg, K., Organic reactions in microemulsions. *European Journal of Organic Chemistry* **2007**, 731-742.
7. Lutz, R.; Aserin, A.; Garti, N., Maillard reaction between leucine and glucose in o/w microemulsion media in comparison to aqueous solution. *Journal of Dispersion Science and Technology* **2005**, *26*, 535-547.
8. Yaghmur, A.; Aserin, A.; Abbas, A.; Garti, N., Reactivity of furfural-cysteine model reaction in food-grade five-component nonionic o/w microemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2005**, *253*, 223-234.
9. Yaghmur, A.; Aserin, A.; Garti, N., Furfural-cysteine model reaction in food grade nonionic oil/water microemulsions for selective flavor formation. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 2878-2883.
10. Yaghmur, A.; de Campo, L.; Aserin, A.; Garti, N.; Glatter, O., Structural characterization of five-component food grade oil-in-water nonionic microemulsions. *Physical Chemistry Chemical Physics* **2004**, *6*, 1524-1533.
11. Yaghmur, A.; Fanun, M.; Aserin, A.; Garti, N., Food grade microemulsions based on nonionic emulsifiers as selective microreactors for flavor formation by Maillard reaction. *Self-Assembly* **2003**, 144-151.
12. Vauthey, S.; Milo, C.; Frossard, P.; Garti, N.; Leser, M. E.; Watzke, H. J., Structured fluids as microreactors for flavor formation by the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2000**, *48*, 4808-4816.
13. Moulik, S. P.; Paul, B. K., Structure, dynamics and transport properties of microemulsions. *Advances in Colloid and Interface Science* **1998**, *78*, 99-195.
14. Ganguli, N. C.; Jain, M. K., Ghee - its chemistry, processing and technology. *Journal of Dairy Science* **1973**, *56*, 19-25.
15. Sserunjogi, M. L.; Abrahamsen, R. K.; Narvhus, J., A review paper: Current knowledge of ghee and related products. *International Dairy Journal* **1998**, *8*, 677-688.

16. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C., Evaluation of volatile compounds in different types of ghee using direct injection with gas chromatography-mass spectrometry. *Journal of Dairy Research* **2002**, *69*, 163-171.
17. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C.; Przybylski, R., Evaluation of volatile compounds in different types of ghee using headspace with GC/MS. *Milchwissenschaft - Milk Science International* **2003**, *58*, 37-39.
18. Yadav, J. S.; Srinivasan, R. A., Advances in ghee flavour research. *Indian Journal of Dairy Science* **1992**, *45*, 338-348.
19. Liu, Y. H.; Friberg, S. E., Perspectives of phase changes and reversibility on a case of emulsion inversion. *Langmuir* **2010**, *26*, 15786-15793.
20. Pasquali, R. C.; Taurozzi, M. P.; Bregni, C., Some considerations about the hydrophilic-lipophilic balance system. *International Journal of Pharmaceutics* **2008**, *356*, 44-51.
21. Akhtar, M.; Murray, B. S.; Dickinson, E., Perception of creaminess of model oil-in-water dairy emulsions: Influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. *Food Hydrocolloids* **2006**, *20*, 839-847.
22. Rawle, A., Basic principles of particle size analysis. In www.malvern.co.uk, Malvern Instruments: www.malvern.co.uk, 2011; pp 1-8.

Chapter 7 Conclusion

The understanding and ability to manipulate the Maillard reaction has the potential to alter the way foods are formulated for cooking. The Maillard reaction has often been to blame for undesirable flavours (1-4) of cooked foods, although the reaction also holds the capability to generate desirable flavours (5). The flavours that are formed in cooked dairy fat emulsion products such as ghee (6, 7) and dulce de leche (8) rely on the Maillard reaction for formation.

While there have been studies into the formation of Maillard reaction compounds (3, 9-14) and into the flavour compounds present in various foods (1, 2, 4, 6, 7, 15-22) along with the impact of certain reaction matrix structures on model reaction systems (9, 10, 23-27), there have been fewer studies investigating the formation of flavour via the Maillard reaction in food (11, 12, 28-32) and how the structure of the food could be used to manipulate the progress of the reaction. The focus of most model systems studies investigating the impact of structure on the Maillard reaction has been through the use of microemulsions (9, 10, 23-27), rather than emulsion structures that are commonly found in food. The study of emulsions has looked mainly at the influence on flavour release and perception (33, 34). This thesis investigated how the fat and emulsion structure of cream influence the generation of flavour via the Maillard and caramelisation reaction and if these parameters could be manipulated to alter the formation of the volatile compound profile.

A SPME/GCMS method was developed and used to identify a range of volatile compounds in a commercial ghee sample and to confirm their presence in traditionally made ghee. The compounds exhibit a range of different flavours and are partially responsible for the flavour of ghee (along with fat related flavour compounds) (6, 7, 17, 18).

The complex nature of cream made it necessary to develop a set of model reaction systems for the caramelisation and Maillard reaction along with a structural model system. The model reaction system was based on lysine and/or lactose and led to the formation of the volatile compounds as identified in the ghee and cooked cream samples. The model system allowed the behaviour of the reactions to be compared in an aqueous system, a two phase system consisting of water and an oil phase and two emulsion structures: an oil in water emulsion and a water in oil emulsion. Parameters associated with each matrix in the structural model system were able to be explored, such as the influence of oil type in a two phase system and the size of oil droplets in the oil in water emulsion structure. It is common knowledge that time and temperature can be used to manipulate the yields of the Maillard reaction; however, a compromise must be reached between the intensity of the flavour and the balance of the desired flavour. The objective of this work was to find new ways of

controlling the Maillard reaction (in addition to time/temperature) that could allow higher yields to be achieved along with a different or more desirable balance of flavours. To validate the model it was important to demonstrate that the model system behaved in the same way as did cream with respect to time and temperature.

The high throughput method of cooking ghee samples that was developed was applied to all cooked cream and model system samples, allowing for control of the time and temperature. Time and temperature were shown to influence the rate of reaction for the model systems and cooked cream/ghee. As the temperature increased so did the yield of volatile compounds; a similar trend was seen as time of cooking was increased.

Changes made to the pH of the starting materials can also provide a method for altering the formation of volatile compounds. Both the caramelisation and Maillard reactions responded to the changes in pH, giving rise to different volatile compound profiles. This confirms that the Maillard and caramelisation reaction are pH dependent (35-40). The manipulation of the pH of cream may be a viable method for the control of flavour formation in ghee if done in a manner appropriate for food.

The most novel aspect of this work for manipulation of volatile compound formation was the manipulation of structure. There has been much interest in the control of the Maillard reaction through the use of different microemulsion structures (9, 10, 23-27, 41-43). These microemulsion structures have allowed the generation of new compounds and control over their formation. The concept of emulsion catalysis via the use of microemulsions to manipulate the Maillard reaction did not translate into an emulsion system. There were, however, several differences found to have been achieved when compounds formed in an aqueous environment were compared with the levels and ratios formed in a two phase system and two emulsion systems.

The addition of fat to an aqueous reaction system to form a two phase reaction matrix consisting of a hydrophilic and a lipophilic layer generated some significant changes in volatile compound formation. The Maillard reaction results indicated a significant increase in the yield of most volatile compounds, while the caramelisation reaction resulted in only a few compounds increasing. This increase in formation indicates that the fat component of food products could be crucial not only because of the intrinsic flavour but also because the fat enhances the development of flavour during cooking. This has the implication that low-fat products could potentially generate less flavour than their full fat counterparts during cooking. The effect of fat on flavour has been investigated in the context of cheese (22, 44) and the impact on sensory properties such as texture and flavour release (45, 46) along with lipid oxidation products participating in the Maillard reaction (39, 47-51).

However, there has not been a lot of research into the role of fat as a structural component of the reaction matrix (52).

Further exploration into the importance of fat in the Maillard and caramelisation reactions indicated that in the model system developed the fat was not participating in the reactions, but there was a change in physical parameters associated with the cooking such as temperature. The temperature was shown early on in the study to be important for the development of flavour, where a difference of 5-10 °C led to significant changes in the levels of various compounds. Internal temperatures of food being cooked are generally lower than the applied external temperatures (53) and are related to the thermal properties of the food. In the case of the model matrix systems, the aqueous matrix did not exceed 100 °C before evaporating, leaving behind solid state products and reactants. The two phase system, however, contains an oil phase that does not evaporate at 100 °C but continued to increase in temperature over the cook time. The increase in temperature is likely a contributor to the progression of the reactions towards higher yields.

There does appear to be some correlation between the increase in compounds with fat addition and the type of fat added to the system. There has been some work conducted to investigate the influence of different fatty acid chain lengths on the Maillard reaction (39, 54). The strong 'buttery' flavour associated with milk fat compared with the mild flavour of canola oil means that differences in flavour formation during cooking with canola oil and milkfat are likely to be insignificant compared with the large flavour difference imparted by the fat before cooking starts.

Following the addition of fat to the system and the conclusion that fat was important for the Maillard reaction but less so for the caramelisation reaction, the emulsion structure was introduced to the reaction system. The introduction of an emulsion structure required the use of emulsifiers including Tween 60 and PGPR, both of which are food grade and already used in food applications. The emulsifiers lead to some surprising results when they were added to the reactions without homogenisation to form the emulsions. Across both the caramelisation and Maillard reactions there was a small change in compound formation with the addition of Tween 60 and a significant decrease in formation with the addition of PGPR, which could be for a variety of reasons, such as micelle formation. This interaction was not explored any further and was used as a baseline for the impact of emulsion structure.

The oil in water emulsion structure mimicked the emulsion structure of cream with discrete droplets of oil throughout the continuous water phase. Formulated with the Tween 60 emulsifier, there were a few compounds that demonstrated a significant decrease in formation; all other compounds

exhibited no significant change. The manipulation of emulsion parameters such as droplet size for the oil in water emulsion did not generate any significant differences; this could be due to coalescence of the droplets during the cooking process prior to the initiation of the caramelisation and Maillard reactions.

The inverted form of the oil in water emulsion is the water in oil emulsion where the discrete water droplets are trapped inside the oil phase. The use of PGPR generated a stable emulsion that led to increased product formation when cooked. This was an interesting development after the decrease in formation seen with the addition of PGPR to the two phase system. Comparison of the results achieved through using the o/w and w/o emulsion allowed the change in ratios to be seen. This change in the ratio of flavour compounds has the potential to provide a change to the final flavour of the product. Not only did the final levels of volatile compounds change but the time at which formation of the compounds began also changed. In the o/w emulsions there were no compounds detected until ~14 minutes; however, in the w/o emulsions product formation was increasing as early as 10 – 12 minutes into the cooking time.

The ability to manipulate the flavour of ghee relies on the ability to manipulate the reactions (Maillard and caramelisation) that are responsible for their formation. These compounds each have a different taste threshold and will contribute differently to the overall product flavour based on this. The ability to change the volatile compound profile of a given sample may not translate to a change in flavour from a sensory perspective. Application of the findings in this thesis from a model system into ghee or other related product for sensory studies would be required to determine the impact of any volatile compound ratio or yield change on the flavour of the product.

There are further avenues that could be investigated for these emulsion structures in relation to the stability of the emulsions. PGPR forms a highly stable emulsion, altering the stability so that the emulsion structure destabilises earlier with cooking and evaporation could yield a different ratio of volatile compounds.

These insights into the importance of emulsion structure can be translated into food systems such as the cooking of ghee. The o/w emulsion system replicates the emulsion structure of cream whereas the w/o structure is similar to that of butter. The issue with the structure of butter for developing flavours in a similar manner to the w/o emulsion is the stability; as the butter heats up and melts the structure is lost. If butter could be structured such that there was a stable emulsion structure present as the fat melted to contain discrete water droplets then the flavour of the ghee generated would change.

Throughout this thesis the objective has been to understand the flavour chemistry occurring in ghee and the influence of emulsion structure on these reactions. Several key findings have been made after the successful development of the analytical method, high throughput cooking method and the model reaction and structure systems. The reaction model was validated against cream and both demonstrated temperature, time and pH dependence as expected. The addition of fat to the matrix gave the largest structure effect and the emulsion structure had a minor effect.

7.1 Future work

There are several areas that could provide avenues towards the generation of different flavours through manipulation of the Maillard and caramelisation reactions.

(1) Influence of fatty acid chain length.

Preliminary experiments as part of this study agreed with recent work that reported different fats and oils could have an influence on the Maillard reaction (54). The impact of the fat and oil properties on flavour formation during the cooking of food could allow for the generation of different flavour types. Different fractions from milkfat could also be studied to identify the best fraction for flavour formation.

(2) Impact of evaporation profiles from different food structures.

There were differences seen in the evaporation profiles of each structural matrix. Further work would allow the investigation into the impact of the rate of evaporation on the Maillard reaction and how the rate of evaporation could be manipulated by altering the food structure.

(3) Stability of emulsions and inversion of emulsions during cooking.

The stability of the emulsion could provide an important tool for manipulating the Maillard reaction in milk fat emulsions. If the emulsion could be broken down or inverted at a specific time or temperature, this could allow different flavours to be formed by changing the emulsion structure during the cooking process.

(4) Development of a method for monitoring the components of the Maillard reaction in ghee during the cooking process in real time.

The ability to monitor the volatile products and intermediate compounds along with reactants and non-volatile compounds would offer a unique insight into the formation of flavour and allow a higher degree of control over the cooking process.

7.2 References

1. Shiratsuchi, H.; Shimoda, M.; Imayoshi, K.; Noda, K.; Osajima, Y., Off-flavour compounds in spray-dried skim milk powder. *Journal of Agricultural and Food Chemistry* **1994**, *42*, 1323-1327.
2. Shiratsuchi, H.; Shimoda, M.; Imayoshi, K.; Noda, K.; Osajima, Y., Volatile flavour compounds in spray-dried skim milk powder. *Journal of Agricultural and Food Chemistry* **1994**, *42*, 984-988.
3. van Boekel, M. A. J. S., Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances* **2006**, *24*, 230-233.
4. Ridgway, K.; Lalljie, S. P. D.; Smith, R. M., Analysis of food taints and off-flavours: a review. *Food Additives and Contaminants Part A - Chemistry Analysis Control Exposure and Risk Assessment* **2010**, *27*, 146-168.
5. Newton, A. E.; Fairbanks, A. J.; Golding, M.; Andrewes, P.; Gerrard, J. A., The role of the Maillard reaction in the formation of flavour compounds in dairy products - not only a deleterious reaction but also a rich source of flavour compounds. *Food and Function* **2012**, *3*, 1231-1241.
6. Yadav, J. S.; Srinivasan, R. A., Advances in ghee flavour research. *Indian Journal of Dairy Science* **1992**, *45*, 338-348.
7. Sserunjogi, M. L.; Abrahamsen, R. K.; Narvhus, J., A review paper: Current knowledge of ghee and related products. *International Dairy Journal* **1998**, *8*, 677-688.
8. Ranalli, N.; Andres, S. C.; Califano, A. N., Physicochemical and rheological characterization of 'dulce de leche'. *Journal of Texture Studies* **2012**, *43*, 115-123.
9. Vauthey, S.; Milo, C.; Frossard, P.; Garti, N.; Leser, M. E.; Watzke, H. J., Structured fluids as microreactors for flavor formation by the Maillard reaction. *Journal of Agricultural and Food Chemistry* **2000**, *48*, 4808-4816.
10. Yaghmur, A.; Aserin, A.; Garti, N., Furfural-cysteine model reaction in food grade nonionic oil/water microemulsions for selective flavor formation. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 2878-2883.
11. Limacher, A.; Kerler, J.; Conde-Petit, B.; Blank, I., Formation of furan and methylfuran from ascorbic acid in model systems and food. *Food Additives and Contaminants* **2007**, *24*, 122-135.
12. Limacher, A.; Kerler, J.; Davidek, T.; Schmalzried, F., Formation of furan and methylfuran by Maillard-type reaction in model systems and food. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 3639-3647.
13. Rizzi, G. P., The Strecker degradation of amino acids: Newer avenues for flavor formation. *Food Reviews International* **2008**, *24*, 416-435.
14. Van Lancker, F.; Adams, A.; Owczarek-Fendor, A.; De Meulenaer, B.; De Kimpe, N., Mechanistic insights into furan formation in Maillard model systems. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 229-235.
15. Yadav, J. S.; Srinivasan, R. A., Qualitative and quantitative changes in flavour characteristics of ghee made from ripened cream. *Indian Journal of Dairy Science* **1984**, *37*, 350-356.

16. Alonso, L.; Juarez, M.; Abd Rabou, N. S.; El-Shibiny, S., Head space analysis of volatile compounds from buffalo and ewe's Samna (ghee) and butter oil. *Milchwissenschaft - Milk Science International* **2002**, *57*, 76-78.
17. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C., Evaluation of volatile compounds in different types of ghee using direct injection with gas chromatography-mass spectrometry. *Journal of Dairy Research* **2002**, *69*, 163-171.
18. Wadodkar, U. R.; Punjrath, J. S.; Shah, A. C.; Przybylski, R., Evaluation of volatile compounds in different types of ghee using headspace with GC/MS. *Milchwissenschaft - Milk Science International* **2003**, *58*, 37-39.
19. McSweeney, P. L. H., The flavour of milk and dairy products: III. Cheese: taste. *International Journal of Dairy Technology* **1997**, *50*, 123-128.
20. Nursten, H. E., The flavour of milk and dairy products. 1. Milk of different kinds, milk powder, butter and cream. *International Journal of Dairy Technology* **1997**, *50*, 48-56.
21. Urbach, G., The flavour of milk and dairy products. 2. Cheese: contribution of volatile compounds. *International Journal of Dairy Technology* **1997**, *50*, 79-89.
22. Drake, M. A.; Miracle, R. E.; McMahon, D. J., Influence of fat on flavour and flavour development in cheddar cheese. *Australian Journal of Dairy Technology* **2010**, *65*, 195-199.
23. Fanun, M.; Leser, M.; Aserin, A.; Garti, N., Sucrose ester microemulsions as microreactors for model Maillard reaction. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2001**, *194*, 175-187.
24. Garti, N., Microemulsions as microreactors for food applications. *Current Opinion in Colloid and Interface Science* **2003**, *8*, 197-211.
25. Yaghmur, A.; Fanun, M.; Aserin, A.; Garti, N., Food grade microemulsions based on nonionic emulsifiers as selective microreactors for flavor formation by Maillard reaction. *Self-Assembly* **2003**, 144-151.
26. Lutz, R.; Aserin, A.; Garti, N., Maillard reaction between leucine and glucose in o/w microemulsion media in comparison to aqueous solution. *Journal of Dispersion Science and Technology* **2005**, *26*, 535-547.
27. Yaghmur, A.; Aserin, A.; Abbas, A.; Garti, N., Reactivity of furfural-cysteine model reaction in food-grade five-component nonionic o/w microemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2005**, *253*, 223-234.
28. Martins, S.; Jongen, W. M. F.; van Boekel, M., A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology* **2000**, *11*, 364-373.
29. Adams, A.; Borrelli, R. C.; Fogliano, V.; De Kimpe, N., Thermal degradation studies of food melanoidins. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4136-4142.
30. Erbersdobler, H. F.; Somoza, V., Forty years of furosine - Forty years of using Maillard reaction products as indicators of the nutritional quality of foods. *Molecular Nutrition and Food Research* **2007**, *51*, 423-430.

31. Mottram, D. S.; Elmore, J. S., Control of the Maillard reaction during the cooking of food. In *Controlling Maillard Pathways to Generate Flavors*, Mottram, D. S.; Taylor, A. J., Eds. 2010; Vol. 1042, pp 143-155.
32. Lineback, D. R.; Coughlin, J. R.; Stadler, R. H., Acrylamide in foods: A review of the science and future considerations. *Annual Review of Food Science and Technology* **2012**, *3*, 15-35.
33. Bayarri, S.; Smith, T.; Hollowood, T.; Hort, J., The role of rheological behaviour in flavour perception in model oil/water emulsions. *European Food Research and Technology* **2007**, *226*, 161-168.
34. Phan, V. A.; Liao, Y. C.; Antille, N.; Sagalowicz, L.; Robert, F.; Godinot, N., Delayed volatile compound release properties of self-assembly structures in emulsions. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 1072-1077.
35. Pauletti, M. S.; Matta, E. J.; Rozycki, S., Kinetics of heat-induced browning in concentrated milk with sucrose as affected by pH and temperature. *Food Science and Technology International* **1999**, *5*, 407-413.
36. Ajandouz, E. H.; Tchiakpe, L. S.; Dalle Ore, F.; Benajiba, A.; Puigserver, A., Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science* **2001**, *66*, 926-931.
37. Ajandouz, E. H.; Desseaux, V.; Tazi, S.; Puigserver, A., Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry* **2008**, *107*, 1244-1252.
38. Yu, A. N.; Zhang, A. D., The effect of pH on the formation of aroma compounds produced by heating a model system containing L-ascorbic acid with L-threonine/L-serine. *Food Chemistry* **2010**, *119*, 214-219.
39. Van Ba, H.; Amna, T.; Hwang, I., Significant influence of particular unsaturated fatty acids and pH on the volatile compounds in meat-like model systems. *Meat Science* **2013**, *94*, 480-488.
40. Nursten, H. E., *The Maillard reaction: chemistry, biochemistry and implications*. RSC Publishing: Cambridge, UK, 2005.
41. Holmberg, K., Organic reactions in microemulsions. *European Journal of Organic Chemistry* **2007**, 731-742.
42. Patel, N.; Schmid, U.; Lawrence, M. J., Phospholipid-based microemulsions suitable for use in foods. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 7817-7824.
43. Yaghmur, A.; de Campo, L.; Aserin, A.; Garti, N.; Glatter, O., Structural characterization of five-component food grade oil-in-water nonionic microemulsions. *Physical Chemistry Chemical Physics* **2004**, *6*, 1524-1533.
44. Johnson, M. E.; Kapoor, R.; McMahon, D. J.; McCoy, D. R.; Narasimmon, R. G., Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. *Comprehensive Reviews in Food Science and Food Safety* **2009**, *8*, 252-268.

45. Relkin, P.; Fabre, M.; Guichard, E., Effect of fat nature and aroma compound hydrophobicity on flavor release from complex food emulsions. *Journal of Agricultural and Food Chemistry* **2004**, *52*, 6257-6263.
46. Prindiville, E. A.; Marshall, R. T.; Heymann, H., Effect of milk fat, cocoa butter, and whey protein fat replacers on the sensory properties of lowfat and nonfat chocolate ice cream. *Journal of Dairy Science* **2000**, *83*, 2216-2223.
47. Capuano, E.; Oliviero, T.; Acar, O. C.; Gokmen, V.; Fogliano, V., Lipid oxidation promotes acrylamide formation in fat-rich model systems. *Food Research International* **2010**, *43*, 1021-1026.
48. Whitfield, F. B., Volatiles from interactions of Maillard reactions and lipids. *Critical Reviews in Food Science and Nutrition* **1992**, *31*, 1-58.
49. Severini, C. R.; Lerici, C., Interaction between the Maillard reaction and lipid oxidation in model systems during high temperature treatment. *Italian Journal of Food Science* **1995**, *7*, 189-196.
50. Adams, A.; Kitryte, V.; Venskutonis, R.; De Kimpe, N., Model studies on the pattern of volatiles generated in mixtures of amino acids, lipid-oxidation-derived aldehydes, and glucose. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 1449-1456.
51. Ho, C. T.; Chen, Q. Y., Lipids in food flavours - An overview. In *Lipids in food flavors*, Ho, C. T.; Hartman, T. G., Eds. American Chemical Society: Washington, DC, United States of America, 1994; Vol. 558, pp 2-14.
52. Potes, N.; Kerry, J. P.; Roos, Y. H., Oil as reaction medium for glycation, oxidation, denaturation, and aggregation of whey protein systems of low water activity. *Journal of Agricultural and Food Chemistry* **2013**, *61*, 3748-3756.
53. Lioumbas, J. S.; Karapantsios, T. D., Evaporation front compared with crust thickness in potato deep-fat frying. *Journal of Food Science* **2011**, E17-E25.
54. Toyosaki, T., Sakane, Y., and Kasai, M., Effects of medium-chain triacylglycerols (MCT) on the Maillard reaction. *Journal of Food Processing and Technology* **2013**, *4*.

Chapter 8 Materials and Methods

8.1 Materials

Cream was purchased fresh on the day of use from the local supermarket with a fat content of ~40 %. Canola oil was also purchased from a local supermarket.

D-Lactose, L-lysine, Tween 60, sodium hydroxide, hydrochloric acid, sodium chloride, sodium dihydrogen phosphate and disodium hydrogen phosphate were all purchased from BDH (Poole, England), Merck (Frankfurt, Germany) or Sigma-Aldrich (St. Louis, MO, United States of America).

Polyglycerol polyricinoleate/Palsgaard 4150 (Palsgaard, Denmark) was gifted by Massey University, Palmerston North, New Zealand.

Anhydrous milk fat (AMF) was supplied by Fonterra Cooperative Group, New Zealand; batch JT16F3872.

All water used was deionised water.

8.2 Gas chromatography mass spectrometry (GCMS)

8.2.1 Vials

Glass Chromacol (Welwyn Garden City, Herts, UK) headspace vials (20 ml) and caps were used for all experiments and analysis.

8.2.2 Reference sample

The reference material used for the development of the method was Dairy Flavour 300 (DF300) which is a commercial ghee sample manufactured by Fonterra. The samples finished product number was 10-920 milk fat: batch 260195: item 12918703. It was manufactured in Stanhope Australia in 2010. The reference sample was a 2.0 ml sample of DF300 in a 20 ml glass headspace vial (8.2.1) with crimped cap. Reference samples were stored frozen (-20 °C). A new reference sample vial was used for each batch of analysis.

8.2.3 Blank sample

The blank sample was a new empty vial as per section 8.2.1 with crimped cap in place. A new blank vial was used for each batch of analysis.

8.2.4 GCMS sample preparation

8.2.4.1 Externally cooked or raw samples

The appropriate amount of the sample was added to the 20 ml headspace vial and sealed with caps containing an intact seal.

Oil 2.0 ml liquid oil

Ghee 2.0 ml liquid ghee

If necessary, samples were warmed to melting in a standard kitchen microwave.

8.2.4.2 Autosampler cooked samples

Samples cooked in the autosampler were cooked with punched caps (section 8.4.2). These caps were removed and replaced with intact caps prior to analysis.

Aqueous samples prepared in the AOC5000 (CTC Combipal, CTC Analytics, Zwingen, Switzerland) autosampler were matrix matched to a standard cream sample by adding 0.8 ml of oil (AMF or Canola oil).

8.2.5 GCMS instrument setup

All analyses used a gas chromatograph (GC) GC2010 (Shimadzu, Kyoto, Japan), coupled with a QP2010 (Shimadzu, Kyoto, Japan) mass spectrometer (MS). The GC column was a 30 m (length) x 0.25 mm (internal diameter) x 0.25 µm (film thickness) EC-Wax column (Alltech Associates, Deerfield, IL, United States of America) with a temperature range of 40 °C – 280 °C (1, 2). The solid phase microextraction (SPME) was automated using the (CTC combipal) robot. The SPME fibre was a 1 cm (length) 23 gauge PDMS-DVB (polydimethylsiloxane/divinylbenzene) fibre (Supelco, Bellefonte, PA, USA). The glass injection liner was split liner 1 mm (internal diameter) x 5 mm (external diameter) x 95 mm (length) for Shimadzu GC (IP deactivated)(3).

The instrument was controlled using Lab Solutions, GCMS solutions software, version 2.5 (Shimadzu, Kyoto, Japan). Peak integration of GCMS chromatograms was also carried out using this software. Compound identification was carried out using the Wiley 7 spectra library as incorporated into the GCMS solutions software.

8.2.6 Experimental GCMS conditions

8.2.6.1 Standard testing conditions

These are the parameters used for all GCMS method development unless otherwise stated (3).

Extraction parameters:	Extraction temperature 60 °C		
	5 min pre incubation		
	20 min extraction onto fibre		
	2 min desorption into injection port		
GC parameters:	Injection temperature	220 °C	
	Carrier gas	helium	
	Flow rate control	linear velocity	
	Linear velocity	37.3 cms ⁻¹	
	Column flow rate	1.06 mlmin ⁻¹	
	Total flow	14.6 mlmin ⁻¹	
	Purge flow	3.0 mlmin ⁻¹	
Injection mode:	Split	1:10 ratio	
MS parameters:	Sampling rate	0.5 (scan)	0.2 (SIM)
	Interface temperature	230 °C	
	Mass range	40 – 350	
	Ion source temperature	200 °C	
	EI	70 eV	

8.2.6.2 Column temperature gradient

The following gradients were run during the preliminary temperature gradient determination. There was a 5 min hold time at 50 °C and again at 220 °C. The sample analysed was a standard DF300

reference sample (section 8.2.2). All other setup for the GCMS conditions is outlined in section 8.2.6.1. Table 8.1 details the initial gradients run and table 8.2 details the second set of iterative gradients. The final temperature gradient is detailed in table 8.3.

Table 8.1: Initial temperature gradients

Gradient (°Cmin ⁻¹)	Start temperature (°C)	Finish temperature (°C)	Time* (min)
1	50	220	180
2	50	220	95
3	50	220	67
4	50	220	53
5	50	220	44
6	50	220	39

*Hold time has been rounded up to the nearest whole minute.

Table 8.2: Second set of iterative temperature gradients

Gradient (°Cmin ⁻¹)	Start temperature (°C)	Finish temperature (°C)	Time* (min)
3	50	220	67
3.25	50	220	63
3.5	50	220	59
3.75	50	220	56
4	50	220	53

*Hold time has been rounded to the nearest whole minute.

Table 8.3: Final temperature profile of GC column

Initial temperature (°C)	Final Temperature (°C)	Rate of change (°Cmin ⁻¹)	Hold time* (min)
50	50	0	2
50	95	6	0
95	95	0	5
95	150	3.25	0
150	170	1	0
170	220	4	0
220	220	0	2

*Hold time has been rounded up to the nearest whole minute.

8.2.6.3 *Injection split ratio*

There were three split ratio conditions that were tested to determine optimum conditions; 1:10, 1:100 and splitless injection. Other GCMS conditions are set out in section 8.3.6.1. The test sample was the standard DF300 sample.

8.2.6.4 *SPME Extraction time*

The time of extraction was tested at 10, 20 and 30 min. Extraction temperature was 60 °C; other conditions are set out in section 8.3.6.1. The test sample was the standard DF300 sample.

8.2.6.5 *SPME Extraction temperature*

Extraction temperatures trialled were 50 °C, 60 °C and 70 °C. Extraction time was 20 min; other conditions are set out in section 8.3.6.1. The test sample was the standard DF300 sample.

8.2.7 Optimised GCMS method

GC setup

Injection temperature	220 °C
Injection mode	split 1:10 ratio
Column start temperature	50 °C
Column end temperature	220 °C
Column temperature gradient	table 8.3
Carrier gas	helium
Flow rate control	linear velocity
Linear velocity	37.3 cms ⁻¹
Column flow rate	1.06 mlmin ⁻¹
Total flow	14.6 mlmin ⁻¹
Purge flow	3.0 mlmin ⁻¹

Mass spectrometer parameters

Sampling rate	0.5 (scan)	0.2 (SIM)
Interface temperature	230 °C	
Mass range	40 – 350	
Ion source temperature	200 °C	
EI	70 eV	

Optimised SPME method

Extraction temperature	60 °C
pre-incubation	5 min
Extraction time	20 min
Desorption time	2 min

8.2.8 Matrix interferences

Analysis of each of the potential matrix interference (4, 5) was carried out on 2.00 ml subsamples. The DF300 was warmed to a liquid state using a standard kitchen microwave.

8.2.8.1 Fat type

A set of aqueous caramelisation and Maillard reaction samples (section 8.5) were cooked (section 8.4.2). Half of the caramelisation and Maillard reaction samples were analysed with canola oil and the other half with AMF.

8.2.8.2 PGPR

PGPR (0.75 g or 1.5 g) was added to 50 ml liquid DF300 and mixed thoroughly.

8.2.8.3 Tween 60

Tween 60 (0.75 g or 1.5 g) was added to 50 ml liquid DF300 and mixed thoroughly.

8.2.8.4 Sodium chloride

Sodium chloride (2.5 g or 5.0 g) was added to 50 ml liquid DF300 and mixed thoroughly.

8.2.8.5 Phosphate buffer

Sodium dihydrogen phosphate (0.45 g or 0.90 g) and disodium hydrogen phosphate (0.35 g or 0.70 g) were added to 50 ml of liquid DF300 and mixed thoroughly.

8.3 General methods

8.3.1 pH measurement

8.3.1.1 Aqueous phase

Aqueous phase pH measurements were made using a Jenway 3510 pH meter (Bibby Scientific Limited, Staffordshire, UK). Calibrations were carried out using pH 4 and pH 7 standards purchased

from Merck (Frankfurt, Germany) and BDH (Poole, England). All pH adjustments were made ± 0.2 pH units. Measurement of pH was carried out between 18 and 22 °C.

8.3.1.2 *Cooked samples*

The pH of samples was measured after cooking by adding 2 ml of DI water and shaking to mix. The pH was measured using Merck pH strips 0-14 (Frankfurt, Germany). Measurement of pH was carried out between 18 and 22 °C.

8.3.2 **Temperature measurement**

Temperature measurements were made using a Fischer Scientific (Waltham, MA, United States of America) traceable thermometer. The probe was inserted into the top of the GC vial and the temperature taken once the temperature had stabilised. The thermometer was suitable for temperature measurements from -50 to + 300 °C.

8.3.3 **Statistical analysis**

Statistical analysis was carried out using Minitab statistical software, version 16 (State College, PA, United States of America) (6, 7).

8.4 **Ghee**

8.4.1 **Wok procedures**

The methods detailed in section 8.4.1 were all cooked in a non-stick Teflon wok (30 cm) on a conventional stove top element. The element was set to temperature mark 4 and stirring was conducted with a non-stick spatula. Stirring was intermittent to prevent sticking of the ghee. The end point was reached when the water had evaporated to leave an oil (ghee) and dark brown solids (melanoidins). The wok was removed from the heat to cool (~ 2 -5 min or <60 °C) and the solids filtered off using a coarse paper towel (3). Each method (8.4.1.1 – 8.4.1.3) was carried out in triplicate. Each replicate was made on a different day using fresh cream. The wok methods are based on traditional ghee methods (8, 9).

8.4.1.1 *Direct cream*

Cream (200 g) was allowed to reach ambient temperature (~ 18 -20 °C) before adding to the wok and heating to form ghee using the method detailed in section 8.4.1.

8.4.1.2 *Cream butter*

Cream (200 g) was churned to butter using a household food processor. Cream was churned until the butter formed a solid mass (~130-135 g) and the excess buttermilk (~60-65 g) was discarded. The butter was transferred to the wok and heated to form ghee using the method detailed in section 8.4.1.

8.4.1.3 *Butter/buttermilk*

Cream (200 g) was churned to butter using a household food processor. Cream was churned until the butter formed a solid mass (~130-135 g) with excess buttermilk (~60-65 g). The butter and buttermilk were transferred to the wok and heated to form ghee using the method detailed in section 8.4.1.

8.4.2 **GC autosampler procedures**

The autosampler on the GC was programmed to cook the ghee samples. The autosampler settings were: temperature, 135 °C; time, 20 minutes; stirring, 500 Hz. The cooking vessel was the headspace vial (section 8.2.1) with a hole punched in the cap seal. All vials had a glass bead added to improve stirring and ensure consistent results. All sample weights are ± 0.1 g. The samples were not filtered prior to analysis.

8.4.2.1 *Direct cream*

Cream (2 g) was weighed into 6 headspace vials.

8.4.2.2 *Cream butter*

Cream (100 g) was churned into ~ 60 g butter as per section 8.8.1.2 (~40 g buttermilk was retained for section 8.8.2.4). Butter (1.3 g) was weighed into 6 headspace vials. The weight of butter was based on measurements from the large scale ghee batch (section 8.8.2.2).

8.4.2.3 *Butter/Buttermilk*

Cream (100 g) was churned into ~ 60 g butter with ~40 g buttermilk as per section 8.8.1.3. Butter (1.3 g) and buttermilk (0.6 g) was weighed into 6 headspace vials. The weight of butter and buttermilk was based on measurements from the large scale ghee batch (section 8.8.2.3).

8.4.2.4 *Buttermilk*

Buttermilk (discarded from 8.8.2.2) was weighed (1.2 g) into 6 headspace vials. The amount was calculated from 60% aqueous volume in cream with 40% fat volume therefore each 2 g of cream was equivalent to ~1.2 g aqueous buttermilk.

8.4.3 Influence of time and temperature

Cream (2.00 ml) was cooked in triplicate. The cook times incrementally increased in 2 minute intervals, starting at 10 minute cooking time and cumulating in a maximum time length of 20 minutes. The experimental temperatures started at 110 °C increasing to a maximum temperature of 135 °C and covered a range of cooking times. The temperature of the sample was measured using a temperature probe in the final minute of cooking. At each of the experimental temperatures triplicate samples of cream were cooked (sample preparation detailed in section 8.8.2.1) at each of the time intervals. The time/temperature experiments were split into two sets.

Experimental set 1 of time/temperature experiments consisted of samples cooked at 110, 120, 125 and 135 °C for 10, 12, 14, 16, 18 and 20 minutes.

Experimental set 2 of time/temperature experiments using smaller temperature differences based on the accuracy of the agitator temperature settings. The same cooking times were used as for experimental set 1 with temperatures of 132, 134, 135, 136 and 138 °C.

8.5 Aqueous experimental model system

The model samples were cooked using the parameters in section 8.4.2 unless otherwise stated. A sample size of 2 ml was used for all samples.

All sample solutions were made up fresh on the day of cooking. The aqueous phase of all samples was pH adjusted to pH 7 using dilute sodium hydroxide unless otherwise stated. The two model reaction systems used were the caramelisation reaction (10, 11) and Maillard reaction (12, 13) as set out in table 8.4 unless otherwise stated. The concentrations are the total concentration of the system. All weights were ± 0.1 g.

Table 8.4 Total reactant concentration for each model system (14)

Reaction system	Lactose (% w/w)	Lysine (% w/w)
Caramelisation	3	0
Maillard	3	2

Caramelisation model samples were made by dissolving 3.0 g of lactose (Fonterra product bulletin, appendix B) in water (total sample weight 100.0 g) at ambient temperature.

Maillard model samples were made by dissolving 3.0 g of lactose and 2.0 g of lysine (Fonterra product bulletin) in water (total sample weight 100.0 g) at ambient temperature.

Cooking was carried out using a 2.0 ml sample.

8.5.1 Influence of time and temperature

The time and temperature influence were assessed using samples set up as per 8.5.1 cooked using the time and temperature data set out in table 8.5 (3, 15-19).

Table 8.5 Time and temperature validation of Maillard and caramelisation reaction samples.

Reaction	Temperature (°C)	Time (min)
Maillard	135	10
Maillard	135	15
Maillard	135	20
Maillard	125	20
Caramelisation	135	10
Caramelisation	135	15
Caramelisation	135	20
Caramelisation	125	20

8.5.2 Changes in pH over time (20, 21)

8.5.2.1 pH changes in cream during cooking

The pH of cream was measured as per section 8.3.1.2. The pH of cream was measured for a series of 2.0 ml cream samples that were cooked for different lengths of time. Each sample was cooked for 2 minutes and 30 seconds longer than the previous sample. The time series ran from time = 0 to time = 20 minutes at intervals of 2 minutes and 30 seconds. All other cooking conditions were as set out in section 8.4.2.

8.5.2.2 pH changes in the Maillard reaction during cooking

The pH of the Maillard reaction was measured as per section 8.3.1.2. The pH was measured for a series of 2.0 ml Maillard model reaction samples (the 2.0 ml samples were taken from a 100 ml solution made up as per section 8.6) that were cooked for different lengths of time. Each sample was

cooked for 2 minutes longer than the previous sample. The time series ran from time = 0 to time = 20 minutes at intervals of 2 minutes. All other cooking conditions were as set out in section 8.4.2.

8.5.3 The influence of pH on the Maillard and caramelisation reactions (15, 17, 18, 22)

The pH of samples was adjusted using HCl (0.5 M) or NaOH (0.1 M).

8.5.3.1 Influence of pH of cream

The pH of cream (25 ml) was adjusted as set out in table 8.6.

Table 8.6 pH adjustment of cream

pH	Adjusted with
3	HCl
5	HCl
7	None required
9	NaOH
11	NaOH

8.5.3.2 Influence of pH on the caramelisation and Maillard reaction

The pH of aqueous caramelisation or Maillard reaction model (100 ml, section 8.5) was adjusted as set out in table 8.7.

Table 8.7 pH adjustment of caramelisation and Maillard model system

pH	Adjusted with
3	HCl
5	None required
7	NaOH
9	NaOH
11	NaOH

8.5.4 Effect of phosphate buffer

Maillard and caramelisation reaction samples were made up as per section 8.5 with the addition of phosphate buffer (0.2 M, pH 7) (23) prior to making the samples up to weight (100.0 g) with water.

Sodium dihydrogen phosphate	0.90 g
-----------------------------	--------

Disodium hydrogen phosphate	0.70 g
-----------------------------	--------

8.5.5 Effect of sodium chloride

Maillard and caramelisation reaction samples were made up as per section 8.5 with the addition of sodium chloride (5%) (24) prior to making the samples up to weight (100.0 g) with water.

Sodium chloride	5.0 g
-----------------	-------

8.6 The addition of fat to the aqueous model system

The ratio for the two phase system was 60% aqueous and 40% fat measured volume for volume (Fonterra product bulletin, appendix B). The concentration of the aqueous phase was increased to adjust for the 60% volume to maintain the concentration as per table 8.4.

Caramelisation model samples were made using an aqueous phase consisting of 5.0 g lactose dissolved in water (total sample weight 100.0 g).

Maillard model systems were made using an aqueous phase consisting of 5.0 g lactose and 3.30 g lysine dissolved in water (total sample weight 100.0 g).

To achieve a two phase system for cooking 1.2 ml of the aqueous phase was added to a headspace vial along with 0.8 ml of fat (canola oil or AMF (25)).

8.6.1 Effect of aqueous concentration

100% aqueous caramelisation and Maillard reaction samples were made up as per section 8.5 and cooked using a 2.0 ml subsample. Samples were cooked as per section 8.4.2.

The aqueous phase of a two phase sample for caramelisation and Maillard reactions were made up as per section 8.6 and cooked using a 1.2 ml subsample. Samples were cooked as per section 8.4.2.

8.6.2 Time series, temperature and evaporation profile

Aqueous Maillard samples were made up as per section 8.5 and two phase samples were made up as per section 8.6. The fat used for the two phase samples was canola oil. The vials were weighed to 4 decimal places before samples were added. The vial and samples were weighed again before being capped for cooking. Samples were weighed after cooking once samples were cool and vial caps were removed.

Samples were cooked for times between 0 and 20 minutes at 2 minute time intervals.

Evaporation of water was calculated using average weights from triplicate cooked samples at each time point. Temperature was measured as per section 8.3.2.

These samples were analysed using GCMS to monitor compound formation.

8.6.3 Participation of fat in the Maillard reaction

A sample was made up as per section 8.6 with the omission of lactose. The fat used for the experiment was canola oil.

8.7 The influence of emulsion structure (26-30)

Emulsions were made with a total volume of 500 ml to be suitable for the homogeniser. The aqueous phase contained 15.0 g lactose and 10.0 g lysine made up with water to 300 g for 60:40 emulsions and 250 g for 50:50 emulsions. The aqueous phase was adjusted to pH 7 with sodium hydroxide (0.5 M).

Emulsifier concentration was 1.50% (w/v) of the total emulsion volume. Tween 60 was added to the aqueous phase and the PGPR to the fat phase. Both phases are equilibrated to 50 °C before homogenisation.

8.7.1 Emulsifier control samples

Each aqueous phase in table 8.8 was made up to 50 g with water. Samples were made for cooking by taking 1.2 ml of the appropriate control sample aqueous phase and adding 0.8 ml of the appropriate fat phase.

Table 8.8 The control samples for the influence of emulsifiers.

Control sample	Lactose (g)	Lysine (g)	Tween 60 (g)	Aqueous phase (g)	Fat phase
Lactose	2.50	0	0	50	Canola oil
Lysine	0	1.65	0	50	Canola oil
Lactose + lysine	2.50	1.65	0	50	Canola oil
Tween 60	0	0	1.25	50	Canola oil
Lactose + Tween 60	2.50	0	1.25	50	Canola oil
Lysine + Tween 60	0	1.65	1.25	50	Canola oil
Lactose + lysine + Tween 60	2.50	1.65	1.25	50	Canola oil
PGPR	0	0	0	50	1.25 g PGPR + 50 ml canola oil
Lactose + PGPR	2.50	0	0	50	1.25 g PGPR + 50 ml canola oil
Lysine + PGPR	0	1.65	0	50	1.25 g PGPR + 50 ml canola oil
Lactose + lysine + PGPR	2.50	1.65	0	50	1.25 g PGPR + 50 ml canola oil

8.7.2 o/w emulsion (influence of emulsion structure and droplet size)

The ratio of the phases for the emulsion system was 60% aqueous and 40% fat measured volume for volume. The contents and weights for the aqueous phase for each reaction model are set out in table 8.9.

Table 8.9 Aqueous phase content for a 60:40 o/w emulsion

Aqueous phase components (g)	Reaction model	
	Caramelisation	Maillard
Lactose	15.0	15.0
Lysine	0.0	10.0
Tween 60	7.5	7.5
Total weight with water	300.0	300.0

After temperature equilibration (50 °C) the emulsion structure was formed by pre-homogenising the emulsion using the ultraturrax. The aqueous phase was mixed at 8500 rpm while the oil phase was slowly added over 30 seconds. The emulsion was then homogenised using a two stage homogeniser.

Different sized droplets were formed using different ultraturrax and homogeniser settings (table 8.10). The emulsion was sampled for cooking and particle size measurements after each stage of homogenisation.

Table 8.10 Stages of homogenisation

Stage	Method	Duration	Speed/pressure
Stage 1	Ultraturrax	2 min	8500 rpm
Stage 2	Ultraturrax	2 min	13500 rpm
Stage 3	Ultraturrax	2 min	20500 rpm
Stage 4	Homogeniser	2 passes	500 psi (first stage) 50 psi (second stage)

8.7.3 Droplet size measurement

The droplet size of emulsions made in section 2.8.2.1 was measured using the Malvern Mastersizer 2000. The method used was adapted from Srinivasan et al (31) using a relative refractive index (N) of 1.095. The ratio of the refractive index of the fat droplet (1.46) to the continuous water phase (1.33). An absorbance value of 0.001 was used.

The droplet size of o/w emulsions was measured using this method. The droplet size of w/o emulsions was not measured because the instrument was not set up for these measurements.

8.7.4 o/w versus w/o (influence of emulsion type)

The aqueous phases (table 8.11) for the emulsions were made up as per table 2.9 and equilibrated at 50 °C along with the corresponding oil phases (table 8.12). These phases were used in a 1 to 1 ratio to generate the appropriate emulsion.

Table 8.11 Phase contents for 50:50 emulsions (Maillard reaction)

Aqueous phase components (g)	Oil in water emulsion	Water in oil emulsion
Lactose	15	15
Lysine	10	10
Tween 60	7.5	0
Total weight with water	250	250

Table 8.12 Oil phase for 50:50 emulsions (Maillard reaction)

Phase	Oil in water emulsion	Water in oil emulsion
PGPR (g)	0	7.5
Total weight (g)	250	250

After temperature equilibration the emulsion structure was formed by pre-homogenising the emulsion using the ultraturrax. The o/w emulsion was formed by mixing the aqueous phase at 8500 rpm while the oil phase was slowly added over 30 seconds. The emulsion was then homogenised using a two stage homogeniser (500 psi/50 psi). The w/o emulsion was formed by mixing the oil phase at 8500 rpm while the aqueous phase was slowly added over 30 seconds. The emulsion was then homogenised using a two stage homogeniser (500 psi/50 psi).

8.7.5 Time series, temperature and evaporation experiments

Emulsions were made up as per section 8.7.4. The vials were weighed to 4 decimal places before samples were added. The vial and samples were weighed again before being capped for cooking. Samples were weighed after cooking once samples were cool and vial caps were removed. Samples were cooked for times between 0 and 20 minutes at 2 minute time intervals.

Evaporation of water was calculated using average weights from triplicate cooked samples at each time point. Temperature was measured as per section 8.3.2.

These samples were analysed using GCMS to monitor compound formation.

8.7.6 Microscopy

Microscopy of the emulsion structure (32) was carried out using an Olympus BX60 light microscope (Tokyo, Japan). Samples were prepared using samples cooked in time series as per section 8.7.2. A droplet of the emulsion was placed on a glass slide and covered with a coverslip. Photos of the emulsions were taken at 80 x magnification.

8.8 References

1. Supelco, GC column selection guide. In www.sigmaaldrich.com, 2013; pp 1-30.
2. DiscoverySciences, Product: GC columns. In www.discoverysciences.com, 2010; pp 216-231.
3. Andrewes, P., Changes in Maillard reaction products in ghee during storage. *Food Chemistry* **2012**, *135*, 921-928.
4. Tsai, S. W.; Kao, K. Y., Determination of furfural in beers, vinegars and infant formulas by solid-phase microextraction and gas chromatography/mass spectrometry. *International Journal of Environmental Analytical Chemistry* **2012**, *92*, 76-84.
5. Leksrisompong, P.; Barbano, D. M.; Foegeding, A. E.; Gerard, P.; Drake, M., The roles of fat and pH on the detection thresholds and coefficients of three compounds: Diacetyl, delta-decalactone and furaneol. *Journal of Sensory Studies* **2010**, *25*, 347-370.
6. Jacobson, D.; Monforte, A. R.; Ferreira, A. C. S., Untangling the chemistry of port wine aging with the use of GC-FID, multivariate statistics, and network reconstruction. *Journal of Agricultural and Food Chemistry* **2013**, *61*, 2513-2521.
7. Minitab, I. *Minitab statistical software*, Release 16 for Windows; State College, Pennsylvania, United States of America, 2009.
8. Ganguli, N. C.; Jain, M. K., Ghee - its chemistry, processing and technology. *Journal of Dairy Science* **1973**, *56*, 19-25.
9. Sserunjogi, M. L.; Abrahamsen, R. K.; Narvhus, J., A review paper: Current knowledge of ghee and related products. *International Dairy Journal* **1998**, *8*, 677-688.
10. Lee, G. C.; Lee, C. Y., Inhibitory effect of caramelisation products on enzymic browning. *Food Chemistry* **1997**, *60*, 231-235.
11. Quintas, M. A. C.; Brandao, T. R. S.; Silva, C. L. M., Modelling colour changes during the caramelisation reaction. *Journal of Food Engineering* **2007**, *83*, 483-491.
12. Fayle, S. E.; Gerrard, J. A., *The Maillard reaction*. Royal Society of Chemistry: Cambridge, UK, 2002.

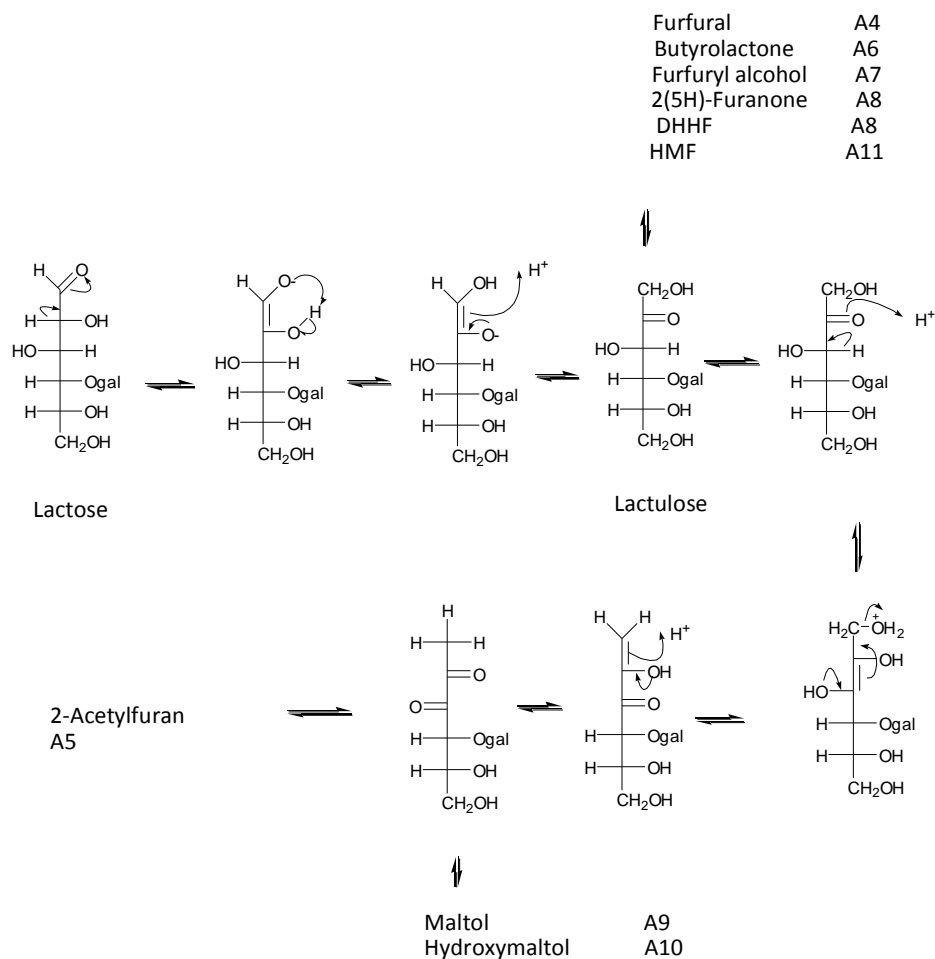
13. Nursten, H. E., *The Maillard reaction: chemistry, biochemistry and implications*. RSC Publishing: Cambridge, UK, 2005.
14. Monti, S. M.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A. L.; Fogliano, V., LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *Journal of Agricultural and Food Chemistry* **1999**, *47*, 1506-1513.
15. Ajandouz, E. H.; Desseaux, V.; Tazi, S.; Puigserver, A., Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry* **2008**, *107*, 1244-1252.
16. Gonzales, A. S. P.; Naranjo, G. B.; Leiva, G. E.; Malec, L. S., Maillard reaction kinetics in milk powder: Effect of water activity at mild temperatures. *International Dairy Journal* **2010**, *20*, 40-45.
17. Parker, J. K., The kinetics of thermal generation of flavour. *Journal of the Science of Food and Agriculture* **2013**, *93*, 197-208.
18. Pauletti, M. S.; Matta, E. J.; Rozycki, S., Kinetics of heat-induced browning in concentrated milk with sucrose as affected by pH and temperature. *Food Science and Technology International* **1999**, *5*, 407-413.
19. van Boekel, M. A. J. S., Kinetic aspects of the Maillard reaction: a critical review. *Nahrung - Food* **2001**, *45*, 150-159.
20. Novotny, O.; Cejpek, K.; Velisek, J., Formation of carboxylic acids during degradation of monosaccharides. *Czech Journal of Food Sciences* **2008**, *26*, 117-131.
21. Smuda, M.; Voigt, M.; Glomb, M. A., Degradation of 1-deoxy-D-erythro-hexo-2,3-diulose in the presence of lysine leads to formation of carboxylic acid amides. *Journal of Agricultural and Food Chemistry* **2010**, *58*, 6458-6464.
22. Ajandouz, E. H.; Tchiakpe, L. S.; Dalle Ore, F.; Benajiba, A.; Puigserver, A., Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science* **2001**, *66*, 926-931.
23. Bell, L. N., Maillard reaction as influenced by buffer type and concentration. *Food Chemistry* **1997**, *59*, 143-147.

24. Rizzi, G. P., Effects of cationic species on visual color formation in model Maillard reactions of pentose sugars and amino acids. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 7160-7164.
25. Toyosaki, T., Sakane, Y., and Kasai, M., Effects of medium-chain triacylglycerols (MCT) on the Maillard reaction. *Journal of Food Processing and Technology* **2013**, *4*.
26. Friberg, S. E.; Larsson, K.; Sjoblom, J., *Food emulsions*. Marcel Dekker Inc: New York, NY, United States of America, 2004.
27. Akhtar, M.; Murray, B. S.; Dickinson, E., Perception of creaminess of model oil-in-water dairy emulsions: Influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. *Food Hydrocolloids* **2006**, *20*, 839-847.
28. Biasutti, M.; Venir, E.; Marchesini, G.; Innocente, N., Rheological properties of model dairy emulsions as affected by high pressure homogenization. *Innovative Food Science and Emerging Technologies* **2010**, *11*, 580-586.
29. Cerimedo, M. S. A.; Iriart, C. H.; Candal, R. J.; Herrera, M. L., Stability of emulsions formulated with high concentrations of sodium caseinate and trehalose. *Food Research International* **2010**, *43*, 1482-1493.
30. Dunstan, T. S.; Fletcher, P. D. I., Compartmentalisation and separation of aqueous reagents in the water droplets of water-in-oil high internal phase emulsions. *Langmuir* **2011**, *27*, 3409-3415.
31. Srinivasan, M.; Singh, H.; Munro, P. A., Influence of retorting (121 degrees C for 15 min), before or after emulsification, on the properties of calcium caseinate oil-in-water emulsions. *Food Chemistry* **2003**, *80*, 61-69.
32. Liu, Y. H.; Friberg, S. E., Perspectives of phase changes and reversibility on a case of emulsion inversion. *Langmuir* **2010**, *26*, 15786-15793.

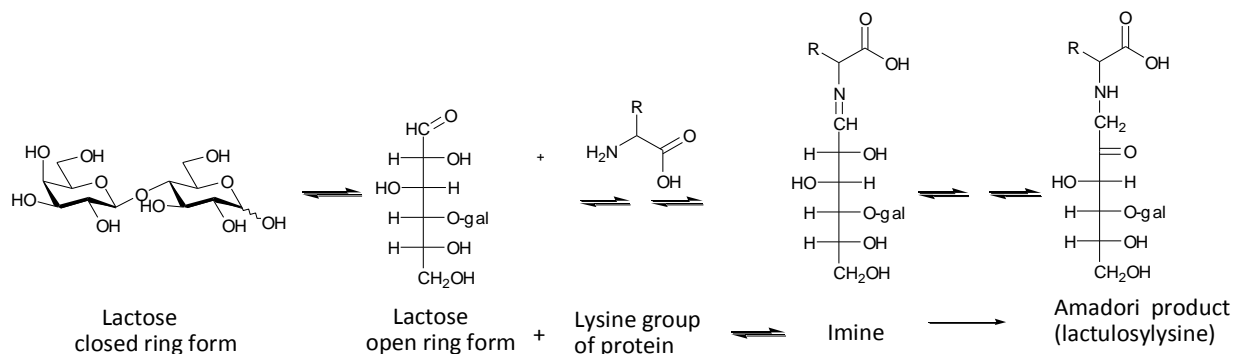
Appendix A

This appendix contains hypothesised mechanisms for the formation of some of the compounds of interest for both the Maillard and caramelisation reactions. Structures for each formed compound can be found in table 2.2 or the respective mechanisms.

A1 Caramelisation reaction

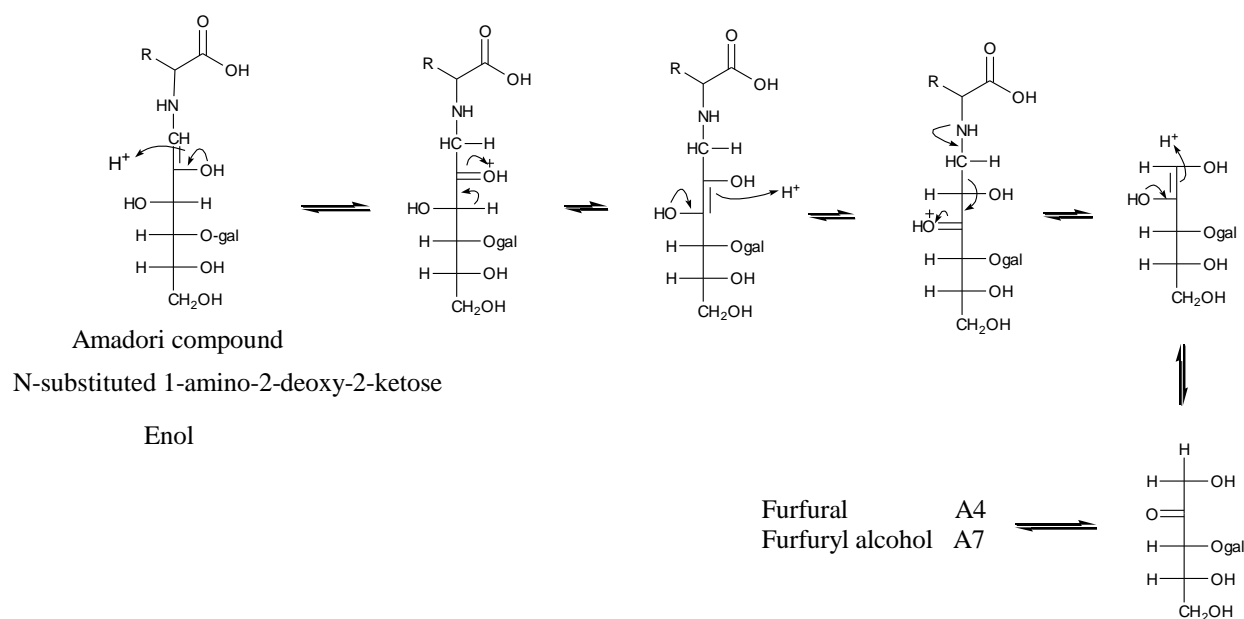


A2 Maillard reaction

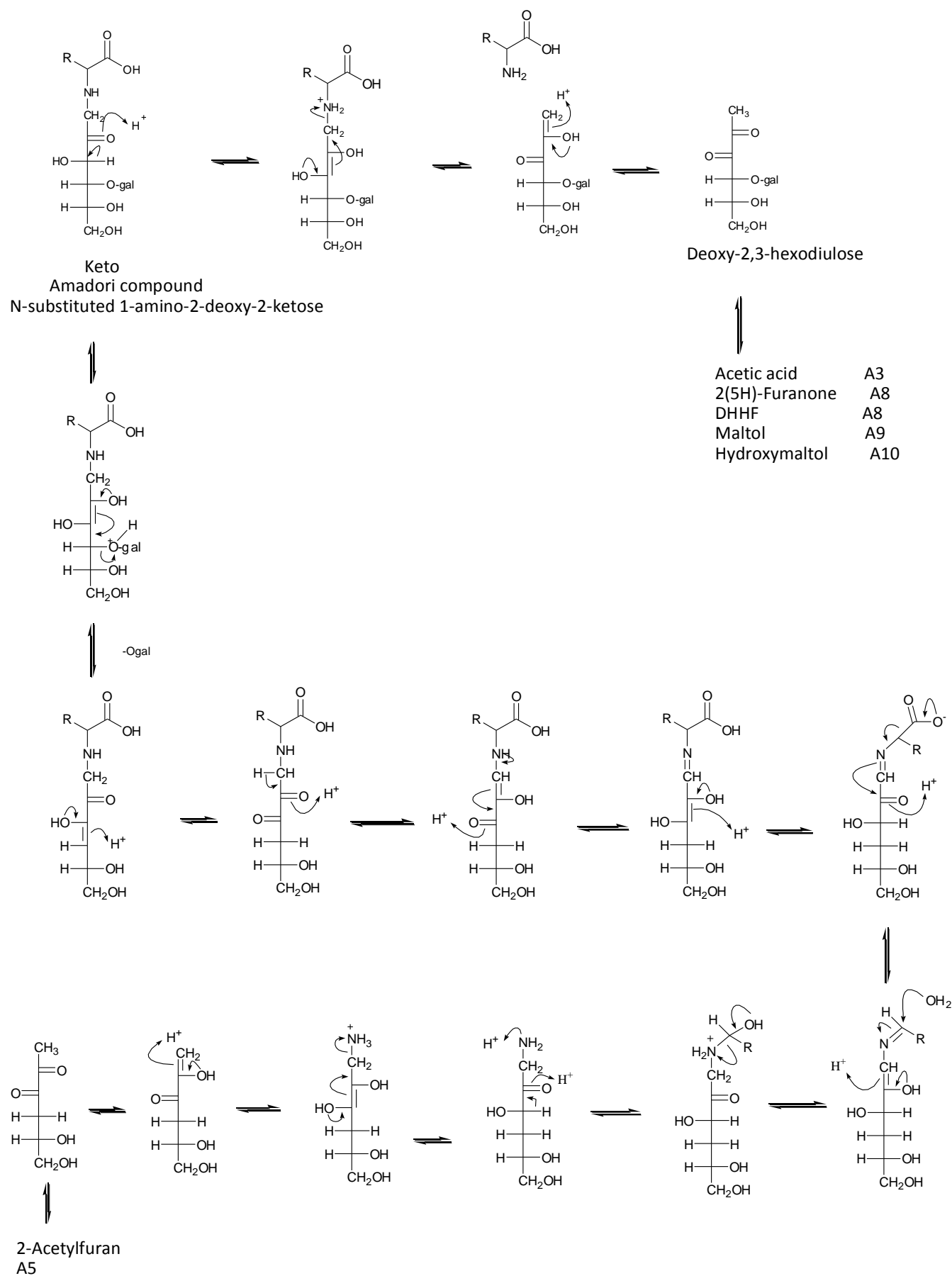


The initial stages of the Maillard reaction, lactose reacts with the N-terminal of the lysine. This is an alternate mechanism to that shown in figure 1.1 where the lactose reacts with the lysine residue. For most compounds the Maillard reaction mechanisms can occur via both the lysine residue and the N-terminal. However, mechanisms occurring via the Strecker degradation such as 2-acetylfuran can only occur when the N-terminal of the amino acid reactions with the lactose. The Amadori compound can then react via a 1,2-enolisation pathway or 2,3- enolisation pathway to form the compounds of interest (table 2.2).

A2.1 1,2-Enolisation reaction pathway from the Amadori product



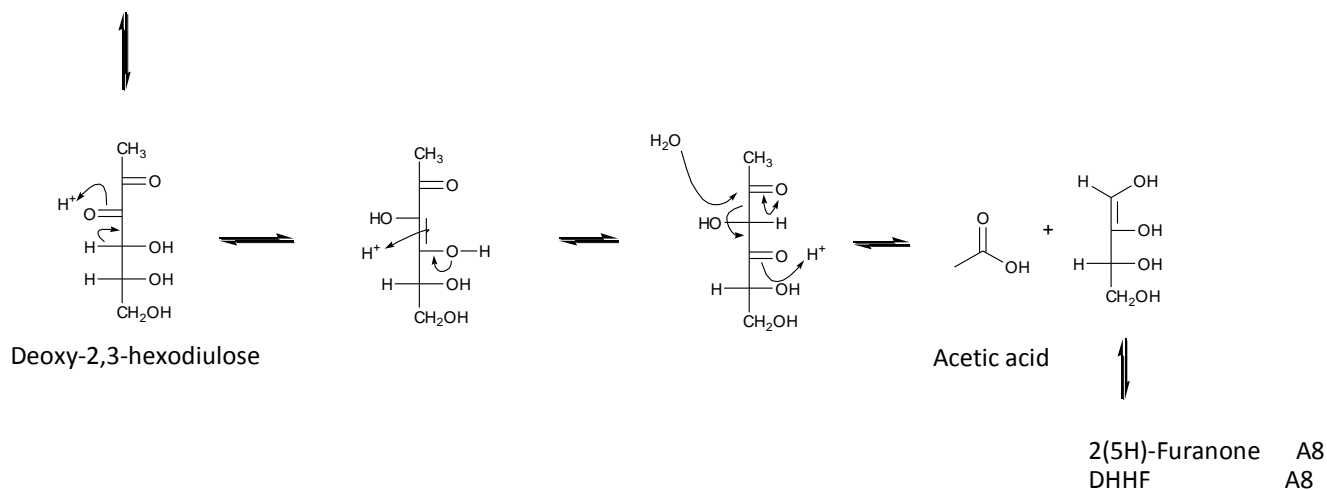
2.2 2,3-Enolisation reaction pathway from the Amadori compound



A3 Acetic Acid

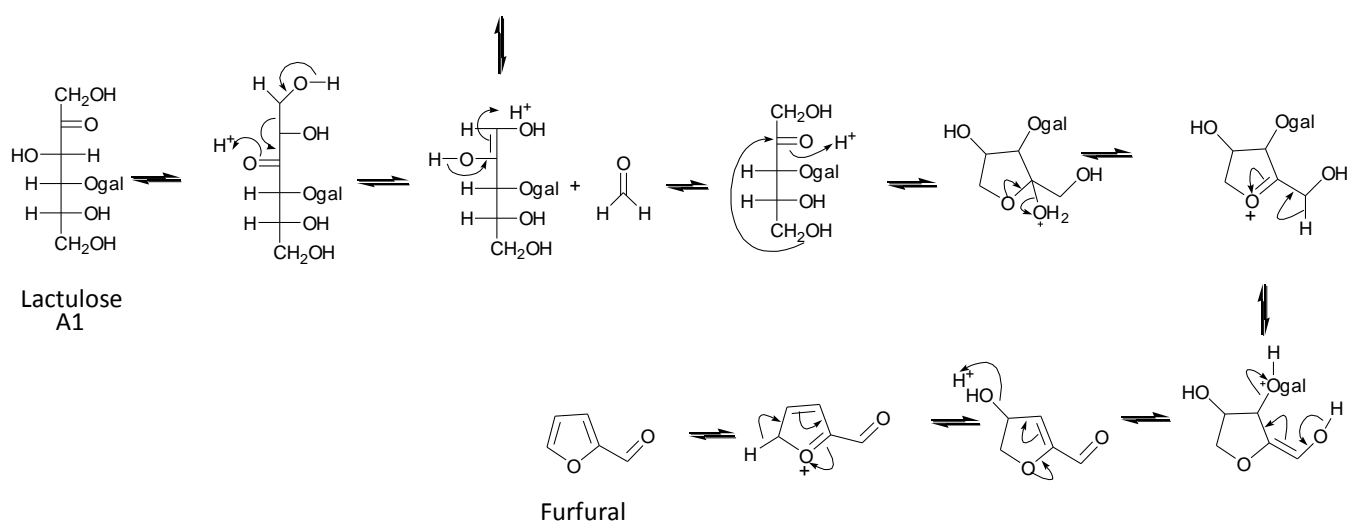
Retro-Claisen condensation reaction

2,3-Enolisation pathway
Maillard reaction
A2.2



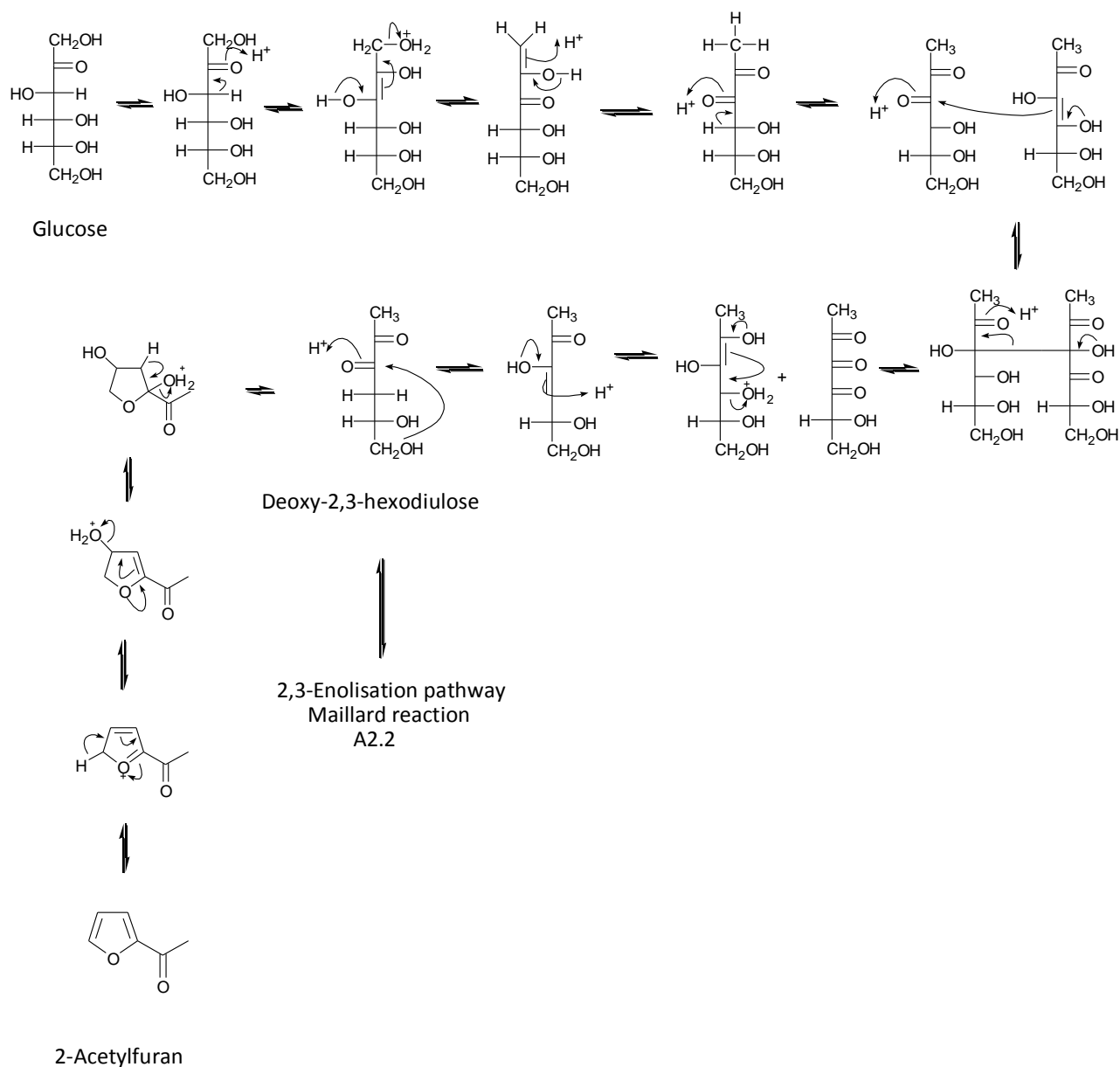
A4 Furfural

1,2-Enolisation pathway
Maillard reaction
A2.1

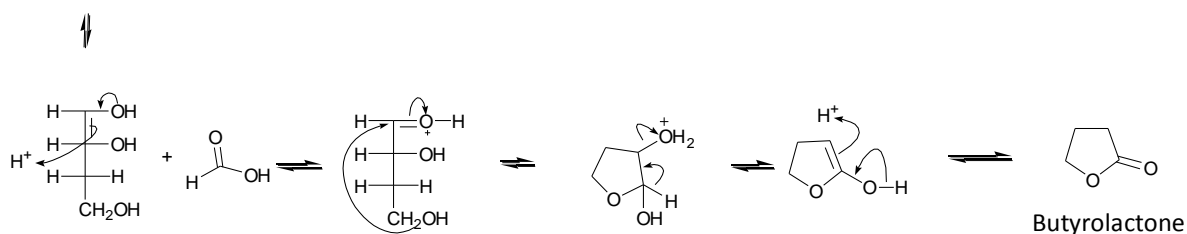
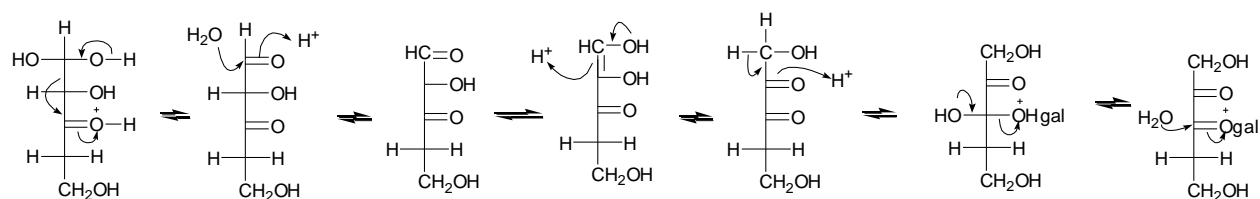
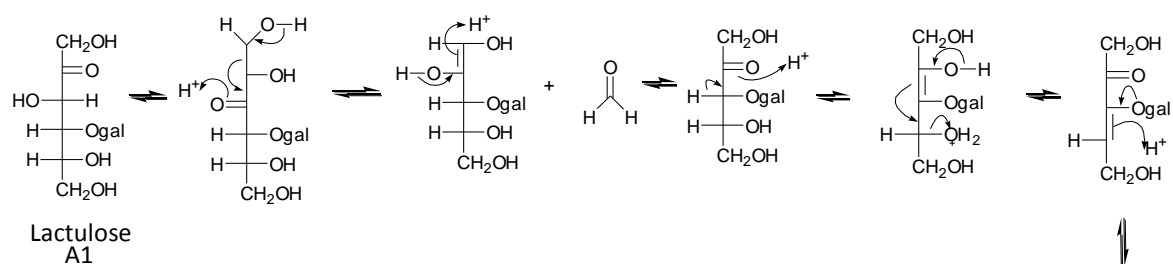


A5 2-Acetylfuran

The caramelisation reaction mechanism occurs via a disproportionation reaction. This mechanism can only happen using glucose or galactose. Lactose is unable to participate in the disproportionation reaction because the presence of galactose at C4.

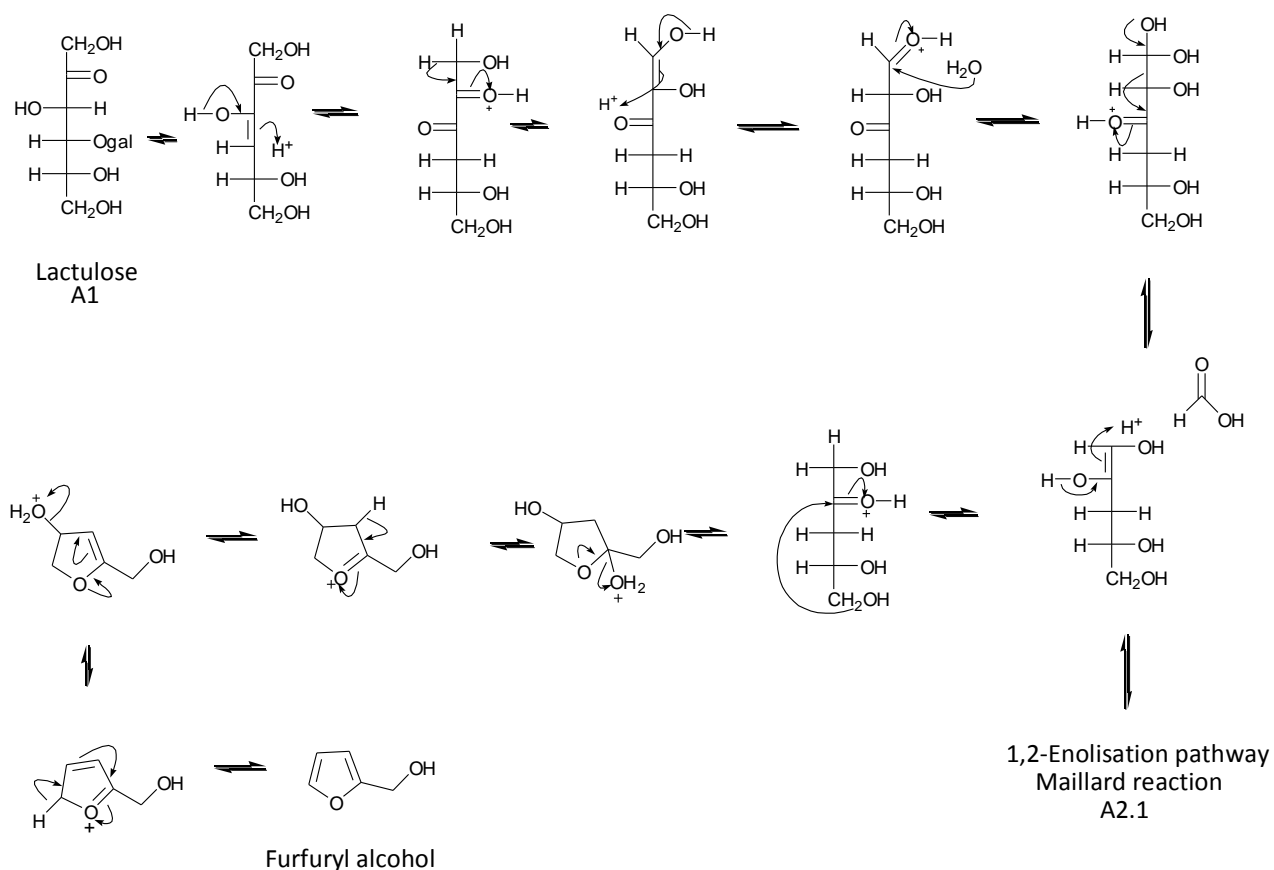


A6 Butyrolactone

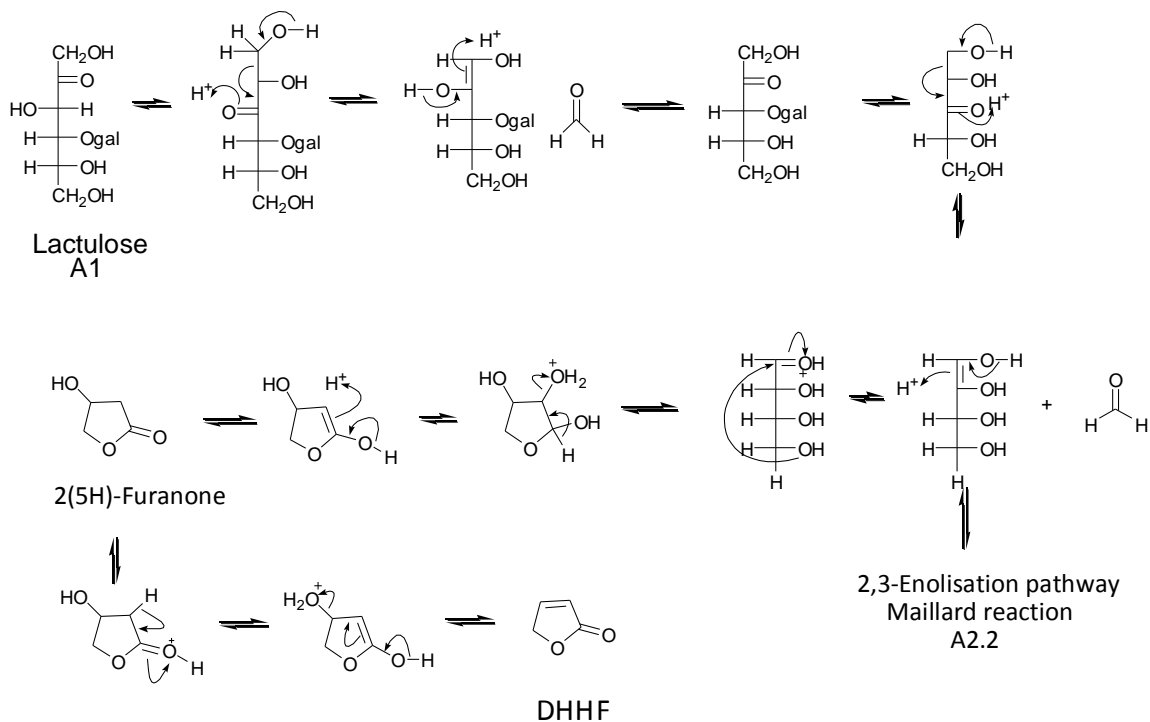


2,3-Enolisation pathway
Maillard reaction
A2.2

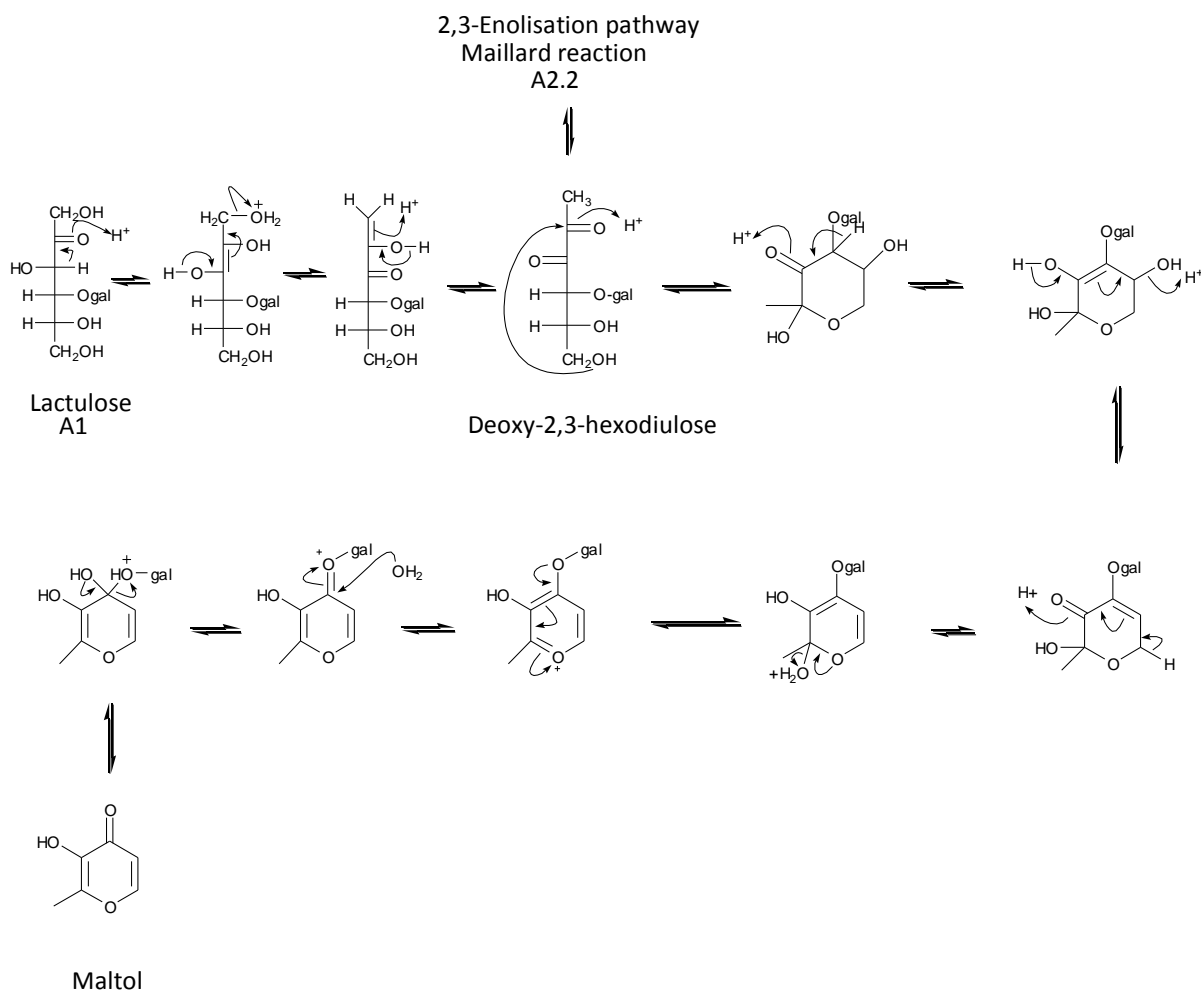
A7 Furfuryl alcohol



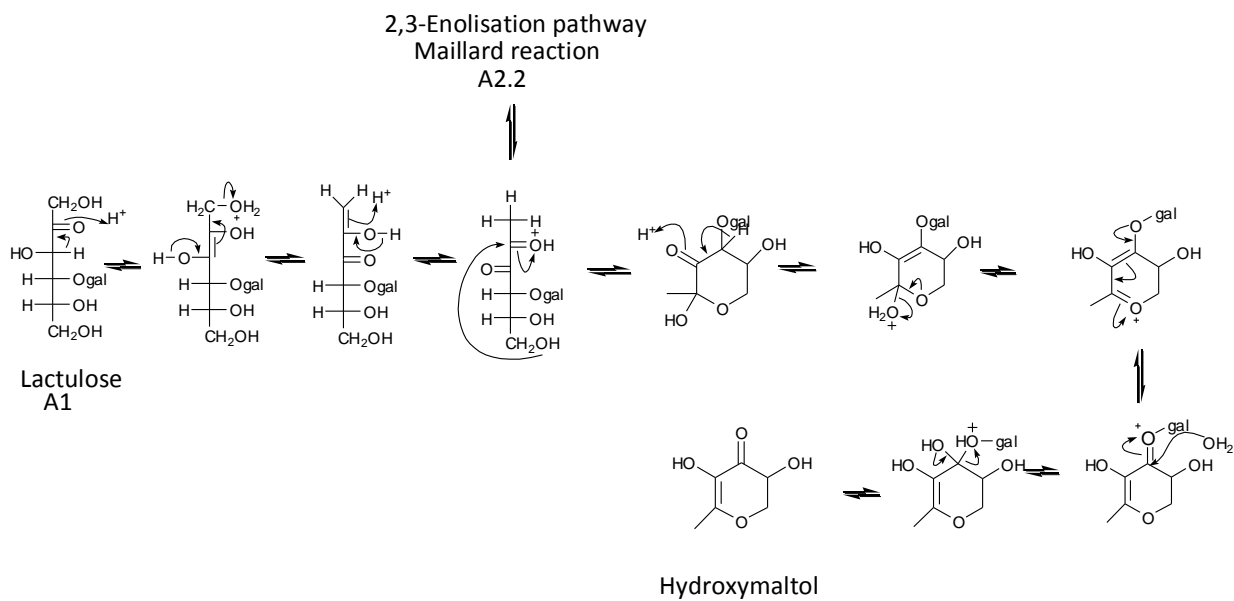
A8 2(5H)-Furanone and Dihydro-4-hydroxy-2(3H)-furanone (DHHF)



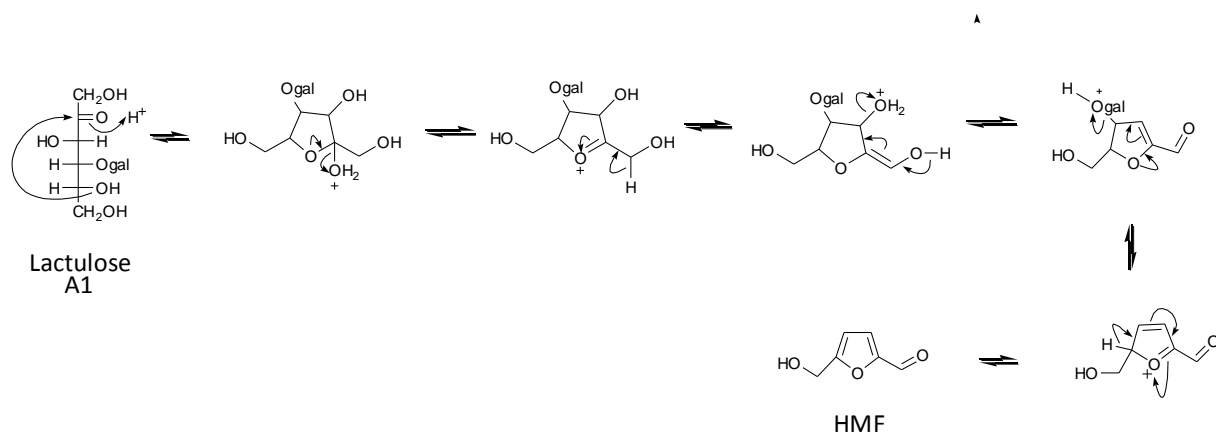
A9 Maltol



A10 Hydroxymaltol



A 11 HMF



Appendix B

This appendix contains the following Fonterra bulletins:

Anhydrous Milk Fat	PB. 102	V. 2.0712
Liquid Cream	PB. 265	V. 1.0511
Typical Amino Acid Profile: Whey Protein Isolate 895	NB. 071	V. 1.0511

Anhydrous Milkfat

Regular Grade

PB.102

Version 2.0712

UNRESTRICTED

Anhydrous Milkfat (regular grade) from Fonterra gives a natural dairy flavour and creaminess to finished products and is the perfect high quality fat ingredient. Anhydrous Milkfat (regular grade) is pure milkfat, derived exclusively from milk and/or products obtained from milk (may include mixtures of cream, butter or whey cream). It has excellent natural keeping qualities. Anhydrous Milkfat (regular grade) is virtually free of moisture and contains no chemical additives, preservatives, flavours, foreign fats or other impurities.

Product Characteristics

- 99.9% pure milkfat made from 100% pure cream.
- Imparts good dairy flavour to product.
- Anhydrous Milkfat (Regular Grade) is full of natural goodness – it contains no additives.
- Is produced in a sophisticated processing plant to ensure product consistency.
- Good shelf life in high quality packaging.

Suggested Uses

- Frying, grilling, roasting
- Sauces
- Recombined dairy products
- Ice cream
- Processed cheese
- Confectionery

Packaging

A round closed-head drum, lined with food grade resin and headspace filled with nitrogen.

Net Weight	210 kg
Gross Weight	226.8 kg
Cubic measure – per drum	0.255 m

Storage and Handling

Anhydrous Milkfat (regular grade) may be transported and stored without refrigeration. However, the storage life will depend on the storage temperature. To maintain quality, it is recommended the product is stored in a cool dry area away from direct sunlight. Provided the seal is unbroken and the above storage conditions are followed, the milkfat will be suitable as a dairy ingredient for at least 12 months after the date of manufacture.

Typical Compositional Analysis

The analysis results listed in this product bulletin are typical. Refer to the selling specification for minimum & maximum limits by parameter.

Fat (g/100g)	99.9
Moisture (g/100g)	<0.1

Typical Mineral Analysis

Iron (mg/kg)	<0.2
Copper (mg/kg)	<0.05
Sodium (mg/100g)	<1
Potassium (mg/100g)	<1
Calcium (mg/100g)	<1

Typical Chemical Analysis

Free fatty acids as Oleic acid (%m/m)	0.2
Peroxide Value (meq O ₂ /kg)	0.2
Iodine value	29 - 39
Reichert Meissl value	22.4 – 31.8
Polenske value	1.5 – 3.4
Kirchner value	17 - 27
Saponification value	225 - 235

Anhydrous Milkfat

Regular Grade

Dairy for life

PB.102

Version 2.0712

UNRESTRICTED

Typical Fatty Acid Composition

(g/100g)	Fatty Acid	Min%	Max %
Butyric	C4:0	2.7	4.5
Caproic	C6:0	1.8	2.7
Caprylic	C8:0	1.0	1.7
Capric	C10:0	2.2	3.9
Lauric	C12:0	2.5	4.5
Myristic	C14:0	9.1	13.1
Myristoleic	C14:1	0.5	1.2
Palmitic	C16:0	23.6	33.3
Palmitoleic	C16:1	0.7	2.3
Stearic	C18:0	9.1	14.6
Oleic	C18:1	18.3	29.6
Linoleic	C18:2	0.8	1.7
Linolenic	C18:3	0.8	2.0
Arachidic	C20:0	0.05	0.3

Density (Typical Seasonal Range)

10°C (kg/dm ³)	0.935 - 0.956
20°C (kg/dm ³)	0.922 - 0.942
30°C (kg/dm ³)	0.909 - 0.925
40°C (kg/dm ³)	0.900 - 0.912
50°C (kg/dm ³)	0.893 - 0.902
60°C (kg/dm ³)	0.888 - 0.893
70°C (kg/dm ³)	0.885 - 0.888

Solid Fat Content

Temperature	Mean	Typical Range
0°C	66.9	58.5 – 70.5
5°	63.3	55.0 – 67.1
10°C	56.4	48.5 – 60.9
15°C	41.0	33.5 – 46.6
20°C	22.5	17.1 – 27.8
25°C	12.4	8.4 – 14.7
30°C	5.9	3.8 – 7.8
35°C	1.0	0.0 – 1.9

Typical Nutritional Analysis

Energy (kJ/100g)	3700
Calories (kcal/100g)	880
Total Fat (Milkfat) (g/100g)	99.9
Moisture (g/100g)	<0.1
Protein (g/100g)	<0.01
Carbohydrate (g/100g)	<0.01
Sugars (Lactose) (g/100g)	<0.01
Dietary Fibre (g/100g)	Nil
Cholesterol (mg/100g)	240
Fatty Acids (g/100g Product)	
Saturated fatty acids	60.2
Total Unsaturated fatty acids	22.6
Mono unsaturated fatty acids	20.6
Poly unsaturated fatty acids	2.1
Trans ¹ fatty acids ²	4.9

¹ Methylene interrupted trans² Please refer to individual country regulations for trans fatty acid labelling requirements

Vitamins

Vitamin A (mg retinol/100g)	1.0
Vitamin A potency (IU/g) (retinol + β carotene)	35 - 50
Vitamin D (mg/100g)	Trace
Vitamin E (mg/100g)	3.8
Vitamin C (mg/100g)	<0.1

Typical Physical Properties

Melting Point (Mettler)	31 - 34°C
Refractive Index	1.4534 - 1.4549
Specific Heat at 40°C (kJ/kg)	2.1
Solubility of water in AMF (40°C)	0.20
Viscosity at 40°C (mPa.s)	31
Viscosity at 50°C (mPa.s)	22



Anhydrous Milkfat

Regular Grade

PB.102

Version 2.0712

UNRESTRICTED

Typical Microbiological Analysis

Aerobic Plate Count (cfu/g)	<100
Listeria (/25g)	Absent
Salmonella (/250g)	Absent
Refer to individual Fonterra product selling specification for specific microbiological limits.	

Quality Assurance

Strict quality control procedures are enforced during manufacture. The manufacturing environment is also subject to regular monitoring and control.

Product is pasteurised.

Final product is sampled and tested for chemical, sensory and microbial parameters using internationally recognised procedures.

During storage and shipment, precautions are taken to ensure that the product quality is maintained. Each package is identified enabling traceback.

Compliance

- Meets CODEX requirements for Anhydrous Milkfat.
http://www.codexalimentarius.net/web/index_en.jsp
- Meets Halal requirements.

Suggested Labelling

Anhydrous Milkfat or AMF

Allergens: Contains Milk and Dairy products

Country regulations for product labelling vary. Fonterra advises customers that they need to check local regulations to determine the correct labelling of this ingredient

Liquid Cream

PB.265

Version 1.0511

UNRESTRICTED

This bulletin applies to both Pasteurised and Unpasteurised Bulk Liquid Cream. Please refer to individual product selling specification.

Product Characteristics

- 100% fresh cream.
- Natural rich flavour imparts premium fresh dairy flavour to product.
- High in fat.

Suggested Uses

- Bulk Liquid Cream is suitable for use in the manufacture of fresh liquid products where a high fat content is required.
- Ice Cream.

Packaging

Delivered in 25000 kg Tankers.

Storage and Handling

Tankers are filled at less than 11°C. Note: Milkfat will crystallize with time after cooling. The process of fat crystallization results in "Heat of Crystallization" being given off. This will result in a rise of temperature of the cream during storage and distribution. Cream should be delivered at 14°C or less.

Typical Compositional Analysis

The analysis results listed in this product bulletin are typical as measured on an "as is" basis. Refer to the selling specification for minimum & maximum limits by parameter.

Milk Solids Not Fat	4.5%
Moisture	54.0%
Fat	41.5%

Typical Nutritional Analysis

Energy (kJ/100g)	1612
Calories (kcal/100g)	384
Protein (g/100g)	2.0
Total Carbohydrate (g/100g)	2.5
Total Sugars (lactose) (g/100g)	2.5
Total Fat (Milkfat) (g/100g)	41.5
Saturated fat (g/100g)	28.9
Sodium (mg/100g)	25

Typical Microbiological Analysis

Aerobic Plate Count (cfu/ml)	< 10,000
Inhibitory Substances (IU/ml)	<0.003
Coliforms (cfu/ml)	<10

Quality Assurance

Strict quality control procedures are enforced during production. The manufacturing environment is subject to strict monitoring.

During storage and shipment, precautions are taken to ensure that the product quality is maintained.

Compliance

- Complies with Food Standards Australia New Zealand (FSANZ) and Animal Products (Dairy) Regulation 2005.

Suggested Labelling

Allergens: Contains Milk and Dairy products

Country regulations for product labelling vary. Fonterra advises customers that they need to check local regulations to determine the correct labelling of this ingredient

Typical Amino Acid Profile

Whey Protein Isolate 895

NB.071 | Version 1.0511 | UNRESTRICTED

The typical value is an average of representative samples taken from across the manufacturing season.

Amino acid

Essential amino acids

Typical Amount
per 100gm of Protein

Isoleucine	6.3 g
Leucine	14.3 g
Lysine	11.2 g
Methionine	2.4 g
Phenylalanine	3.8 g
Threonine	5.3 g
Tryptophan	2.4 g
Valine	5.6 g

Non-Essential amino acids

Histidine (Essential for Infants)	2.0 g
Alanine	5.7 g
Arginine	3.0 g
Aspartic acid	12.5 g
Cysteine/cystine	4.0 g
Glutamic acid	17.6 g
Glycine	1.8 g
Proline	4.5 g
Serine	4.5 g
Tyrosine	4.2 g

Principle Components Analysis (PCA)

Principle components analysis was carried out using Minitab software (version 16) (1). The PCA generates linear combinations of the original data that was entered into the system. A set of principle component (PC) values is calculated for each variable, indicating how far the variable is from the calculated axis. The axis is the linear combination that describes the variation across all of the variable responses and has a calculated Eigenvalue corresponding to the amount of variation explained by that axis; the higher the Eigenvalue the higher the percentage of variation explained by that axis (1).

For the experiments in this thesis, the peak areas of the eleven monitored compounds were entered for each individual sample. The principle components were calculated using a correlation matrix in which all the variables (peak area) had been standardised. The peak areas were standardised because each compound has a different response factor, compounds with a very large or very small response factor can skew the data. The standardisation is also appropriate because it is the relative amounts of compounds present rather than the absolute quantities in each sample. The linear combinations (principle components) explain the given amount of variance within the data set (1).

The PCA score plots represent each sample along with similarities and dissimilarities between them. The x and y axis of the PCA score plot represent the first two principle components calculated to explain the variation within the samples (1). A loading plot can be used in combination with the score plot to identify which variables are associated with which samples.

Samples that are similar to each other with respect to the variables represented using the principle components (peak area of the monitored compounds) will be clustered close together while samples with a lesser degree of similarity will be further apart. The percentage of variance explained by each principle component as plotted on the PCA score plot give an indication of the degree of similarity between samples: a large PC value would indicate that the first principle component explains a large percentage of variation and therefore samples spread along this axis exhibit large differences; however, a small PC value indicates only a small percentage of variation is explained and therefore samples spread along the axis exhibit only small differences (1).

Analysis of Variance (ANOVA)

An ANOVA using Minitab software (version 16) was employed in the study/thesis (1). This analysis was used to investigate the relationship between changes in responses (eleven monitored compounds) and changes in sample treatments (pH, time, temperature, matrix structure). The calculation investigates whether the means of the groups are, statistically, the same or different (1). The null hypothesis is that the means of the groups are statistically the same. The alpha value (α) is the level of confidence you have in correctly rejecting the null hypothesis when it is true. The p-value relates to the chances of saying that there is a significant relationship when in fact there is none, i.e. a p-value of 0.05 means that 95 times out of 100 we will correctly assume that there is an effect, but 5 times out of 100 we will say there is an effect when there isn't one.

To test for significance a p value of <0.05 was used; any variation with a p value less than 0.05 was regarded as indicating a reliably significant difference in the results. This equates to a 95% confidence interval, indicating that there is 95% confidence in the means being different. Along with calculating the p value an F value was also calculated, the F value is the ratio of the explained (can be attributed to the experimental factors) and unexplained (attributed to error) variances in the experiment. A large F value indicates that variance has been efficiently explained and when coupled with a small p value provides strong evidence that the results are significantly different from one another (1).

Other information that can be obtained from the ANOVA includes interactions between treatments of data, and the main effects of the treatment for each response. Prior to the ANOVA calculations the normality of the data was tested. It is important that the data points are normally distributed for each response, in this case for each compound. For all ANOVA carried out in this study the residuals were checked and all showed normal distributions. The interaction plots give an insight into the complex relationships occurring between treatments and the main effects show the effect of a single factor acting alone on the samples (1).

Supplementary ANOVA tables

Table C1 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the differences between aqueous (Aq) and two phase (TP) matrices for the Maillard reaction.

Compound	Mean (Aq)	Mean (TP)	F value	p value
Furfural	2200	24000	3.23	0.09
Acetic acid	100000	150000	200.00	0.00
2-Acetylfuran	110000	210000	6.88	0.02
Butyrolactone	2600	2800	22.60	0.00
Furfuryl alcohol	98000	550000	160.00	0.00
2(5H)-Furanone	130000	320000	20.62	0.00
Maltol	0	60000	5.67	0.03
2-Acetylpyrrole	36000	19000	26.63	0.00
Hydoxymaltol	220000	220000	0.00	0.97
HMF	4900	9000	8.89	0.01
DHHF	94000	90000	0.07	0.79

Table C2 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the Maillard reaction showing the significance of differences between AMF and canola oil.

Compound	Mean (AMF)	Mean (Canola)	F value	p value
Furfural	29000	50000	294.22	0.00
Acetic acid	200000	200000	0.02	0.90
2-Acetylfuran	460000	460000	0.00	0.98
Butyrolactone	11000	13000	8.30	0.02
Furfuryl alcohol	67000	110000	119.19	0.00
2(5H)-Furanone	520000	590000	1.63	0.23
Maltol	75000	35000	2.14	0.17
2-Acetylpyrrole	300000	220000	4.96	0.05
Hydoxymaltol	390000	220000	12.83	0.01
HMF	5300	7500	1.21	0.30
DHHF	170000	120000	3.75	0.08

Table C3 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the significance of differences between aqueous (Aq) and two phase (TP) matrices for the Caramelisation reaction.

Compound	Mean (Aq)	Mean (TP)	F value	p value
Furfural	1800	3400	5.47	0.03
Acetic acid	5400	4900	0.03	0.87
2-Acetylfuran	1700	2200	0.89	0.36
Butyrolactone	1200	700	6.79	0.02
Furfuryl alcohol	1700	10000	6.82	0.02
2(5H)-Furanone	5600	12000	21.59	0.00
Maltol	Not detected	Not detected		
2-Acetylpyrrole	Not detected	Not detected		
Hydroxymaltol	Not detected	Not detected		
HMF	3600	2900	0.28	0.60
DHHF	5900	3700	3.87	0.06

Table C4 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the caramelisation reaction showing the significance of differences between AMF and canola oil.

Compound	Mean (AMF)	Mean (Canola)	F value	p value
Furfural	3000	6100	40.98	0.37
Acetic acid	6900	0	0.89	0.00
2-Acetylfuran	4100	4700	1.50	0.25
Butyrolactone	1300	700	16.15	0.00
Furfuryl alcohol	20000	10000	7.89	0.02
2(5H)-Furanone	15000	7000	21.64	0.00
Maltol	Not detected	Not detected		
2-Acetylpyrrole	Not detected	Not detected		
Hydroxymaltol	Not detected	Not detected		
HMF	1400	700	1.97	0.19
DHHF	2900	2500	0.47	0.51

Table C5 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the effect of aqueous concentration on the Maillard reaction.

Compound	Mean (100% Aq)	Mean (60% Aq)	F value	p value
Furfural	2200	2400	0.36	0.55
Acetic acid	110000	150000	5.22	0.03
2-Acetylfuran	110000	190000	3.77	0.07
Butyrolactone	2700	3400	2.05	0.16
Furfuryl alcohol	97000	82000	3.76	0.07
2(5H)-Furanone	130000	190000	5.33	0.03
Maltol	Not detected	Not detected		
2-Acetylpyrrole	36000	59000	3.04	0.10
Hydroxymaltol	220000	200000	0.14	0.71
HMF	4700	4100	0.95	0.34
DHHF	94000	140000	13.31	0.00

Table C6 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the effect of aqueous concentration on the caramelisation reaction.

Compound	Mean (100% Aq)	Mean (60% Aq)	F value	p value
Furfural	1800	2000	0.26	0.62
Acetic acid	5400	4900	0.07	0.80
2-Acetylfuran	1700	700	6.11	0.02
Butyrolactone	1200	1600	6.31	0.02
Furfuryl alcohol	17000	16000	0.00	0.96
2(5H)-Furanone	5600	5800	0.03	0.86
Maltol	Not detected	Not detected		
2-Acetylpyrrole	Not detected	Not detected		
Hydroxymaltol	Not detected	Not detected		
HMF	3600	1700	2.31	0.15
DHHF	5900	4900	0.74	0.40

Table C7 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the difference between o/w emulsion and two phase Maillard reaction.

Compound	Mean (o/w)	Mean (TP)	F value	p value
Furfural	40000	35000	0.63	0.47
Acetic acid	420000	520000	12.18	0.02
2-Acetylfuran	280000	710000	2.80	0.00
Butyrolactone	13000	15000	4.30	0.11
Furfuryl alcohol	1300000	1000000	2.41	0.20
2(5H)-Furanone	170000	290000	3058.45	0.00
Maltol	1300000	3000000	75.36	0.00
2-Acetylpyrrole	110000	490000	174.81	0.00
Hyoxymaltol	310000	710000	144.20	0.00
HMF	11000	18000	23.70	0.01
DHMF	140000	290000	69.59	0.00

Table C8 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for differences between different droplet sizes in o/w emulsions for the Maillard reaction.

Compound	Means				F value	p value
	A*	B*	C*	D*		
Furfural	40000	40000	40000	40000	0.01	0.99
Acetic acid	420000	850000	720000	860000	4.81	0.02
2-Acetylfuran	280000	87000	720000	19000	3.17	0.06
Butyrolactone	13000	19000	18000	19000	1.20	0.35
Furfuryl alcohol	1300000	140000	1400000	1400000	0.04	0.99
2(5H)-Furanone	170000	390000	370000	390000	3.24	0.060
Maltol	1300000	2500000	2500000	2500000	16.25	0.000
2-Acetylpyrrole	110000	310000	320000	330000	17.82	0.000
Hyoxymaltol	310000	650000	650000	680000	7.39	0.01
HMF	11000	10000	10000	16000	6.87	0.00
DHMF	140000	280000	270000	280000	35.78	0.00

*A = homogeniser; B = 20,000 ultraturrax; C = 13500 ultraturrax; D = 8500 ultraturrax.

Table C9 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the difference between o/w emulsion and two phase caramelisation reaction.

Compound	Mean (o/w)	Mean (TP)	F value	p value
Furfural	23000	17000	10.78	0.08
Acetic acid	36000	83000	152.33	0.01
2-Acetylfuran	8800	5400	61.71	0.02
Butyrolactone	4700	6400	7.57	0.12
Furfuryl alcohol	150000	90000	472.00	0.00
2(5H)-Furanone	60000	73000	2.05	0.89
Maltol	Not detected	Not detected		
2-Acetylpyrrole	Not detected	Not detected		
Hydroxymaltol	Not detected	Not detected		
HMF	1800	2100	3.99	0.18
DHMF	15000	7700	122.30	0.01

Table C10 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for differences between different droplet sizes in o/w emulsions for the caramelisation reaction.

Compound	Means				F value	p value
	A*	B*	C*	D*		
Furfural	23000	31000	28000	27000	4.66	0.09
Acetic acid	36000	53000	46000	3900	77.00	0.00
2-Acetylfuran	8800	8600	7500	7400	0.94	0.50
Butyrolactone	4800	8400	7400	7100	23.02	0.01
Furfuryl alcohol	150000	250000	210000	200000	45.53	0.00
2(5H)-Furanone	61000	64000	78000	5900	1.36	0.37
Maltol	Not detected	Not detected	Not detected	Not detected		
2-Acetylpyrrole	Not detected	Not detected	Not detected	Not detected		
Hydroxymaltol	Not detected	Not detected	Not detected	Not detected		
HMF	1800	3100	2400	2200	3.73	0.12
DHMF	15000	13000	12000	8300	6.98	0.05

A = homogeniser; B = 20,000 ultraturrax; C = 13500 ultraturrax; D = 8500 ultraturrax.

Table C11 Means, ANOVA F and p values ($p < 0.05$ = significant reliable difference) for the difference between o/w and w/o emulsion structures for the Maillard reaction.

Compound	Mean (o/w)	Mean (w/o)	F value	p value
Furfural	40000	70000	611.02	0.00
Acetic acid	270000	96000	10.04	0.01
2-Acetylfuran	280000	890000	42.19	0.00
Butyrolactone	11000	17000	32.82	0.00
Furfuryl alcohol	110000	1000000	6.15	0.04
2(5H)-Furanone	370000	870000	2.80	0.13
Maltol	440000	960000	2.58	0.15
2-Acetylpyrrole	170000	440000	8.32	0.02
Hydroxymaltol	160000	390000	1.93	0.20
HMF	47000	160000	1.31	0.29
DHHF	120000	230000	181.45	0.00

References

1. Minitab, I. *Minitab statistical software*, Release 16 for Windows; State College, Pennsylvania, United States of America, 2009.